ANNALS OF BOTANY

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The Genus Sphaeroplea.[1]

BY

F. E. FRITSCH.

With eight Figures in the Text.

(*a*) Introductory Remarks.

THE extensive group of the Siphonales is by many authorities classed in the two series Siphoneae and Siphonocladeae (Blackman and Tansley (1), Oltmanns (16), Printz (17)), the latter comprising the forms in which more or less complete septation occurs. The numerous members of the Siphonocladeae are variously grouped, and the difficulties of classification are apparent as soon as one becomes somewhat more familiar with the types involved. The writer has always felt that many of the forms placed in the Siphonocladeae are only of very doubtful affinity with the strictly siphoneous type, and has recently removed the Cladophoraceae and Sphaeropleaceae from the former and placed them among the Ulotrichales (West and Fritsch, 22). A suggestion in this direction was made by Oltmanns (15, p. 14) in the first edition of his algal work, but was not followed up in the second. Some of the many reasons for referring Cladophoraceae to the Ulotrichales have been given elsewhere (22, p. 147), and it is not proposed to go into this matter here. The object of the present communication is to deal with the genus *Sphaeroplea*, the only member of Sphaeropleaceae, of which the writer has had the opportunity of examining very extensive material belonging to various species from different parts of South Africa, as well as from two localities in England.

The most usual habitat of *Sphaeroplea* is periodically inundated ground (Cohn, 3, p. 188; Rauwenhoff, 18, p. 93), and in South Africa, too, it favours the temporary 'vleis', which are only under water during the wet season. It is perhaps owing to the abundance of suitable habitats that the genus is so well represented in this part of the world. It has, however, also been encountered rather frequently in artificial waters (Fritsch, 5, p. 198; Heinricher, 11, p. 433). Printz (17, p. 298; cf. also Skuja, 20, p. 37) records its occurrence in slightly brackish water, and the locality in which Professor Lloyd Williams found *S. cambrica* in Carnarvonshire is likewise

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among sand-hills in the neighbourhood of the sea, although no exact details as to the character of the water are available. It may be pointed out that most of the habitats in which species of this genus occur naturally are of a rather extreme type, which may either imply adaptation on the part of these Algae to very exceptional conditions, or point to an inability to compete successfully in more favourable situations. At the same time the rather frequent appearance in well-aerated water (fountains, springs) may indicate a preference for situations in which the requisite gases are easily obtained. It would appear that the period of vegetative activity is often short (cf. Lowe, 13, p. 37), though relatively long in *S. africana* var. *crassa*.

(*b*) The Vegetative Structure.

(1) *The cell-wall and septa.* The unbranched threads, which are frequently of great length, are not uncommonly somewhat constricted at the septa, so that the segments become slightly barrel-shaped (cf. Fig. 2 A). There is great variety in the length of the individual segments, occasional ones being quite short (two–three times as long as broad, Fig. 2 E), the majority much longer and usually from about twenty to thirty-five times their breadth, whilst Rauwenhoff (18, p. 95) records some reaching a length of as much as ninety times their breadth; but in most species this is very exceptional. In *S. tenuis*, however, both septa and 'plugs' (cf. p. 6) are commonly at very remote intervals, and lengths of upwards of 1000 μ may often be found (threads 8·5–15 μ wide) without any interruption of the continuity of the cell-contents. The width of the threads in most species ranges between 20 and 50 μ, although in *S. africana* var. *crassa* the threads are much wider (diam. 112–170 μ), and in *S. tenuis* they range from 8·5 to 15 μ, being most usually 10–12 μ wide. In all cases the longitudinal walls and the septa give a pronounced cellulose-reaction.

The longitudinal walls are always comparatively thin, sometimes indeed strikingly so, in relation to the breadth of the thread, and in view of the infrequent septa it is difficult to understand how any considerable degree of turgor pressure can be met. At the same time, the septa are often exceedingly thick and elaborate, but this is not always the case; especially in some of the narrower forms of *S. annulina*, they may not be appreciably thicker than the longitudinal walls (Fig. 2 B). The septa are practically always circular in outline.

The peculiar thick septa produced by this genus were first described by Heinricher (11) in a form of *S. annulina*, found in an artificial basin at Graz; a few years later Rauwenhoff (18) dealt with them in material from the same source. Heinricher's form has generally been regarded as a distinct var. *crassisepta*. Both investigators describe the formation of the septa by gradual ingrowth of a homogeneous annular rudiment, but emphasize that *in many cases* the septum remains incomplete with a more or less irregular

central aperture through which the contents of the adjacent segments communicate freely (11, t. xii, Fig. 1 d; 17, t. iv, Fig. 16). Heinricher (p. 434) also draws attention to the fact that septum-formation may commence at different points, not all lying in one plane perpendicular to the long axis of the thread. 'Trotzdem kommt es indess in der Regel doch zu einem Verschluss, indem die einzelnen Wandteile in der Mitte der Zelle verwachsen,

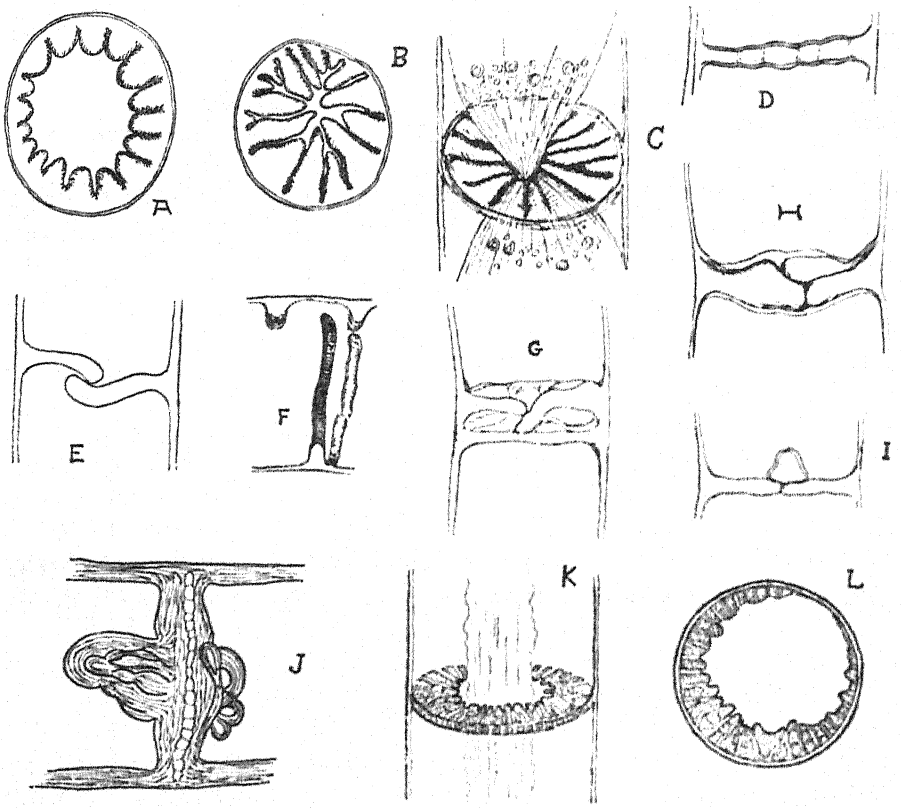


FIG. 1. Septa of *S. africana*, Fritsch (A–I) and of *S. africana* var. *crassa* nov. var. (J–L). In A–C, K, and L the septa are seen from the surface, in the other cases in optical section. Full description in the text. (A–I × 500; J–L × 200).

respective verklebt werden.' He is apparently referring to the kind of thing depicted by Rauwenhoff on t. iv. Fig. 17, and both the description and the figure imply, though there is no definite statement to that effect, that in these cases the septum is composed of a number of separate ingrowing processes, as in *S. africana* and its var. *crassa*, now to be described.

In this species (cf. also Fritsch, 6) the septa usually originate as a continuous ring-shaped strip of membrane apposed to the longitudinal walls (Figs. 1 A and L). Instead of this initial ring exhibiting uniform ingrowth, as apparently occurs in the other species, separate finger-like processes now

arise in the case of *S. africana* and grow more or less regularly towards the centre, meeting together to form a more or less complete septum (Fig. 1, K, B, C, H). In the variety *crassa* there are far more of these processes than in the case of the type itself (Fig. 1 K and L). It is owing to this peculiar method of formation of the septum that the latter in optical section presents a beaded, somewhat moniliform appearance (Fig. 1 D and J). Whether a complete separation of the adjacent protoplasts is effected by such septa is doubtful, and one can certainly find many cases, like those depicted in Fig. 1 C and K, in which the protoplasm though constricted continues without interruption through the septum. Sometimes the processes are all more or less equal, but in other cases they are of varied length and breadth, and only some reach the centre (Fig. 1 B). It has not been possible to settle whether the finger-like processes fuse laterally or whether narrow interspaces persist between them; there is much to suggest that the latter is often the case (cf. Fig. 1 C), but the point could only be settled finally by investigation of fresh material. Heinricher (Fig. 1 d on Table XII) shows a marginal ring with a few ingrowing processes, but as above pointed out his account suggests that this is a very exceptional condition and not the rule as in *S. africana.*

In many cases secondary thickening layers are deposited on either side of the septum thus produced (Fig. 1 D), and in *S. africana* var. *crassa* such thickening may be very extensive (Fig. 1 J) and lead to the formation of the exceedingly thick septa often found in this form. It appears that simultaneously thickening layers are also deposited on the inner side of the longitudinal walls, although the thickness of the latter is never at all comparable to that of the septa. These thickening layers in *S. africana* var. *crassa* often show prominent stratification with rather irregular strata (Fig. 1 J).

Apart from the normal septa just described, numerous irregularities are to be found in *S. africana.* Very often the processes do not grow in a regular radial manner towards the centre, but overlap and become entangled with one another in various ways (Fig. 1 E, G, H). A not uncommon condition is that in which some of the processes, either only on one or on both sides of the septum, after growing inwards in a radial direction, turn out at right angles, leading to the development of a central hump (Fig. 1 I). This was observed particularly frequently in the var. *crassa*, and, when further thickening layers are deposited around these outstanding processes, an exceedingly bizarre appearance may result (cf. Fig. 1 J). These structures recall those found in *S. annulina* var. *crassisepta* (11, p. 434, t. xii, Fig. 1 a–c), where, however, Heinricher interprets them as cellulose-knobs deposited on one or both sides of the septum, a matter requiring further investigation.

Other frequent anomalies in *S. africana* consist in the development of successive processes from the longitudinal walls, more or less close together,

without forming a proper septum (Fig. 1 F); probably the very short segments occasionally found (Fig. 2 E) result from this kind of development. Similar isolated processes are known in *S. annulina* var. *crassisepta* (11, p. 434, t. xii, Fig. 2; 18, p. 114, t. iv, Fig. 17, 19). Short knob-like in-

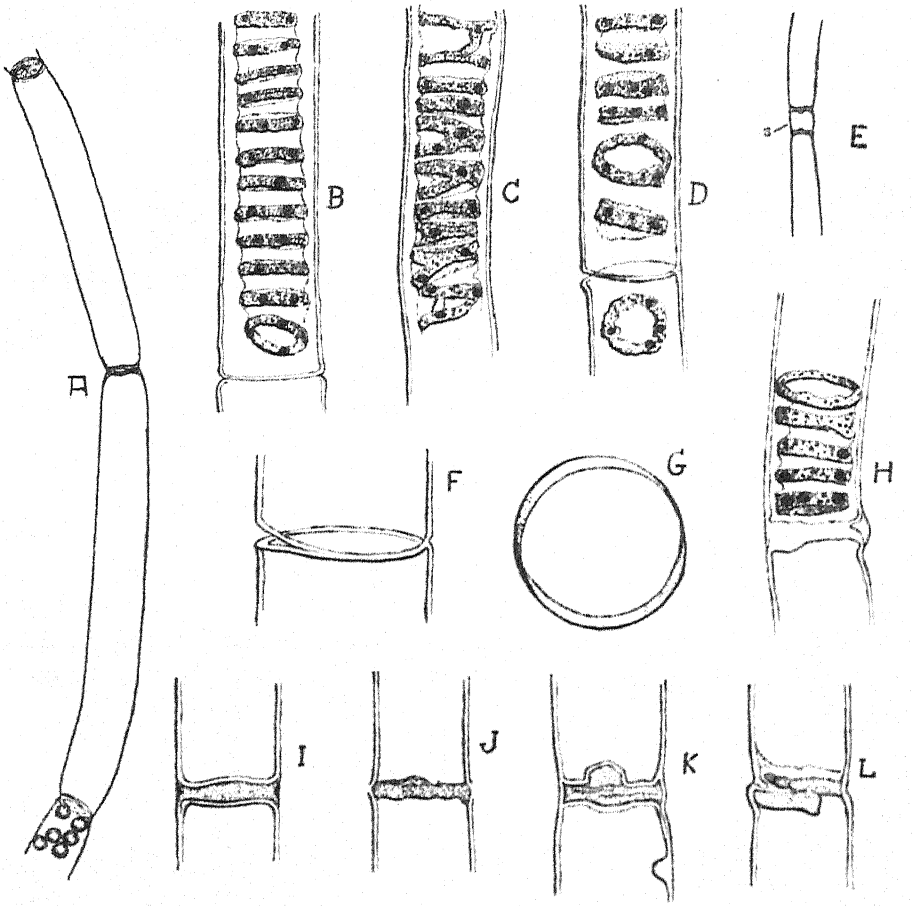


FIG. 2. A, small part of thread of *S. africana*, Fritsch. B–D, *S. annulina* (Roth), Ag. showing the annular chloroplasts and stages in their division (pyrenoids dark). E, short segment of *S. africana*. F and G, septa of *S. annulina*, normal form. H, *S. wilmani*, Fritsch and Rich, chloroplasts. I–L, the same, septa. (A and E × 150; B–D × 450; F, G × 800; H × 425; I–L × 640).

growths, of a similar nature to the processes, are to be encountered on the longitudinal walls at many points in both species (cf. Fig 1 F), and are occasionally found in *S. wilmani* (Fig. 2 K).

The rather thick septa of this latter species (cf. also (7)) appear to be homogeneous and not built up of separate processes (Fig. 2 I and J), although occasional anomalies (Fig. 2 L), as in *S. annulina* var. *crassisepta*, indicate a tendency in that direction. There is, indeed, much correspondence between the septa of the two forms. The thick middle part of the

septum in *S. wilmani* is stratified to some extent,[1] whilst the secondary thickening layers on either side, which are continuous with the inner layer of the longitudinal walls, remain comparatively thin. There is a great tendency for rupture of the threads at the septa, and this appears to be initiated by the break-down of the outer layer of the longitudinal walls at the level of the septum (Fig. 2 J). Such fragmentation evidently also occurs commonly in *S. annulina* var. *crassisepta* (cf. 11, p. 436). The septa of *S. cambrica*, though often relatively thin, appear to be of much the same type.

In the ordinary form of *S. annulina* the septa are thin circular discs like those of many other Algae (Fig. 2 F, G), though usually with a rather definite marginal rim which is continued a little way on to the longitudinal wall above and below the septum, giving a highly refractive appearance to the wall at these points. Here again splitting at the septum is a common phenomenon (Fig. 2 F).

In *S. tenuis* true septa are comparatively rare. They appear as a general rule to be biconvex discs which remain relatively thin, although large lumps of a whitish opaque-looking material are often found on one (Fig. 4 D, M) or both (Fig. 4 I) sides of the septum. Fig. 4 G shows a case where the septum has undergone thickening by the deposition of secondary thickening layers. Far commoner than these septa are more or less extensive plugs of white opaque-looking matter, apparently of the same nature as the masses found on the septa. These plugs may form anywhere in the threads, and may be quite short (Fig. 4 E, F, H) or of very considerable length (Fig. 4 J, K, N), sometimes exceeding 100 μ. Their ends are often quite irregular (Fig. 4 E, K, N), although usually sharply marked off from the adjacent cell-contents. Sometimes the material composing the plugs is homogeneous, at other times it shows irregular stratification (Fig. 4 E, F).

The plugs stain readily with methylene blue, gentian violet, &c., but not with haematoxylin or sharlack red. Strong sulphuric acid does not affect them more rapidly than the longitudinal walls, and they are not dissolved by potash. With iodine and chlor-zinc-iodide they assume a more or less brownish colouration. Within the plugs part of the occluded cell-contents can always be recognized. Pyrenoids are invariably present, and still take on a deep stain with iodine reagents (Fig. 4 E, F, K); in the elongate plugs these pyrenoids usually lie in a single series (Fig. 4 J, K), but they are sometimes very numerous and quite irregularly distributed (Fig. 4 N). Sometimes part of the cell-contents, in addition to the pyrenoids, is to be recognized, and in such cases there often seem to be transverse gaps in the substance of the plug (Fig. 4 J). It seems clear that the formation of these plugs centres around the chloroplasts, sometimes involving only one, some-

[1] There are some indications that this part of the septum in *S. wilmani* continues to increase in thickness after the formation of the secondary layers on either side, and this may possibly be responsible for the rupture of the longitudinal walls at this level.

times a number. These peculiar occlusions of the cell-contents of *S. tenuis* seem to represent yet another way of attaining septation in this genus.

It would appear from the above considerations that *Sphaeroplea* produces septa of two types; in *S. annulina*, *S. cambrica*, *S. tenuis*, and *S. wilmani*, they are usually homogeneous discs, whilst in *S. africana* they are complex and composed of a series of radial processes. At the same time in those forms in which thick septa occur (*S. annulina* var. *crassisepta*, *S. wilmani*) there is an occasional production of irregular processes. In these, moreover, the septa frequently remain incomplete,[1] and this is a very marked feature in *S. africana* and its var. *crassa*. It may be suggested that normal septation is disappearing altogether in the genus *Sphaeroplea* and being replaced by groups of cellulose bars, somewhat comparable to those of a *Caulerpa*. The ordinary form of *S. annulina* shows normal cross-walls, and in its var. *crassisepta*, as well as in *S. cambrica* and *S. wilmani*, many of the septa are practically normal in type, and certainly bring about a complete closure; but in the variety of *S. annulina* with thick septa many remain incomplete, and here we find, too, a frequent development of cellulose processes. Most advanced in both respects is *S. africana*, where normal septa appear to be lacking, although closure is still effected in many cases by the secondary thickening layers. It is worthy of comment that the form in which these features are most pronounced, *S. africana* var. *crassa* possesses by far the widest threads as yet known in this genus, whilst the type itself is wider than the usual forms of *S. annulina*, &c. A detailed study of the mode of growth of the septa in fresh material of *Sphaeroplea* may be recommended as likely to afford data of general interest. The remarkable plugs of *S. tenuis* may well be another substitute for septa which in this species are often few and far between.

(2) *The cell-contents.* There has been much confusion about the chloroplasts of *Sphaeroplea*, and no feature has contributed more to obscure the true affinities of the genus. The original description of the chlorophyll-apparatus of *S. annulina* by Braun (2, p. 164) is so true to fact that it may well be cited in the first place: 'The distribution of the contents is extremely elegant and allows, in some measure, of a comparison with that in *Spirogyra*, with this difference, however, that the chlorophyll forms, not spiral, but annular bands, twenty or thirty of which girdle the internal cavity as green zones, in each cell. In the median line of these zones are found, as in the spiral bands of *Spirogyra*, starch-vesicles, from five to seven in each ring; the surface of the rings, moreover, appears spotted with very fine granules, and the margins irregularly denticulated. The outer surface of the zones lies flat upon the cell-wall, the inner surface projects with a strong keel into the cavity. The whole series of zones are connected together by a delicate, colourless, mucilaginous layer or primordial

[1] This has not yet been established for *S. wilmani*.

membrane, which lines the whole of the internal surface of the cell-membrane and which is traversed by extremely delicate lines and branched and anastomosing green longitudinal streaks issuing from the teeth of the zones'. Schmitz (19, p. 13) likewise interprets each ring as a single structure (cf. also Fresenius 4, p. 242, t. vi, Fig. 1, 13, 14; Meyer 14, p. 61).

In 1899, however, Klebahn (12, p. 85), in a special study of the fertilization process of this form of *S. annulina*,[1] described each of the green annular zones as being composed of a large number of irregularly-shaped chloroplasts of diverse size arranged to form a network, each ring including three to ten pyrenoids 'probably enclosed in larger chromatophores'. Adjacent rings are stated not rarely to be connected by rows of small chloroplasts or by broader 'gitterartige Streifen' (cf. also loc. cit., p. 94; and Fresenius 4, p. 243, Figs. 4 and 8). These observations were made on fixed and stained material.

Klebahn's interpretation of the chloroplasts has been universally accepted, so much so that in many cases the normal appearance of the unstained thread is not depicted at all (see Oltmanns 16, p. 383; Heering 10, p. 67; Printz 17, p. 297), a striking outcome of the obsession for cytological detail. There can be no doubt, however, that in the ordinary vegetative thread of *S. annulina* there are a series of annular chloroplasts with smooth or more or less denticulate margins, and containing a limited number of large pyrenoids in a single series (Fig. 2 B–D). This is the impression I obtained when examining fresh material of *S. annulina* from the tank near the Jodrell Laboratory at Kew many years ago, and the South African material of this species shows the fact so diagrammatically that no one could question the correctness of the interpretation. In this condition, too, one finds little in the way of connexions between consecutive rings, although stages in their division or reticulation are not uncommon. Forked rings, such as are shown in Fig. 2 C, are by no means rare (cf. also Fresenius 4, t. vi, Fig. 1), and the longitudinal connexion between the two uppermost rings in this figure is no doubt merely a further stage in the separation of the two halves. *S. wilmani* has quite similar annular chloroplasts (Fig. 2 H), often rather more delicate than those of *S. annulina*. It is possible, though not very probable, that investigation of stained and carefully-fixed material might disclose a detailed structure of these rings, similar to that described by Klebahn, but that would not alter the fact that each ring behaves as a separate entity and is to be regarded as one single chloroplast.

The number of separate rings may be very considerable in the elongate segments. Rauwenhoff (18, p. 97) records as many as seventy. On the other hand, Gutwinski (8, p. 108) has described a form with very short segments (41–81 μ long; 50 μ wide) containing only one or two broad chloroplasts (11–34·5 μ wide) in each cell.

Stages in the reticulation of the rings, such as that shown in Fig. 3 C,

[1] Often known as *S. annulina* var. *braunii*.

are occasionally met with, and it is probable that this condition may at certain times (and perhaps especially in certain forms of *S. annulina*) lead to the development of a somewhat diffuse network, approaching that characteristic of *S. africana*. It is not impossible that such reticulation may

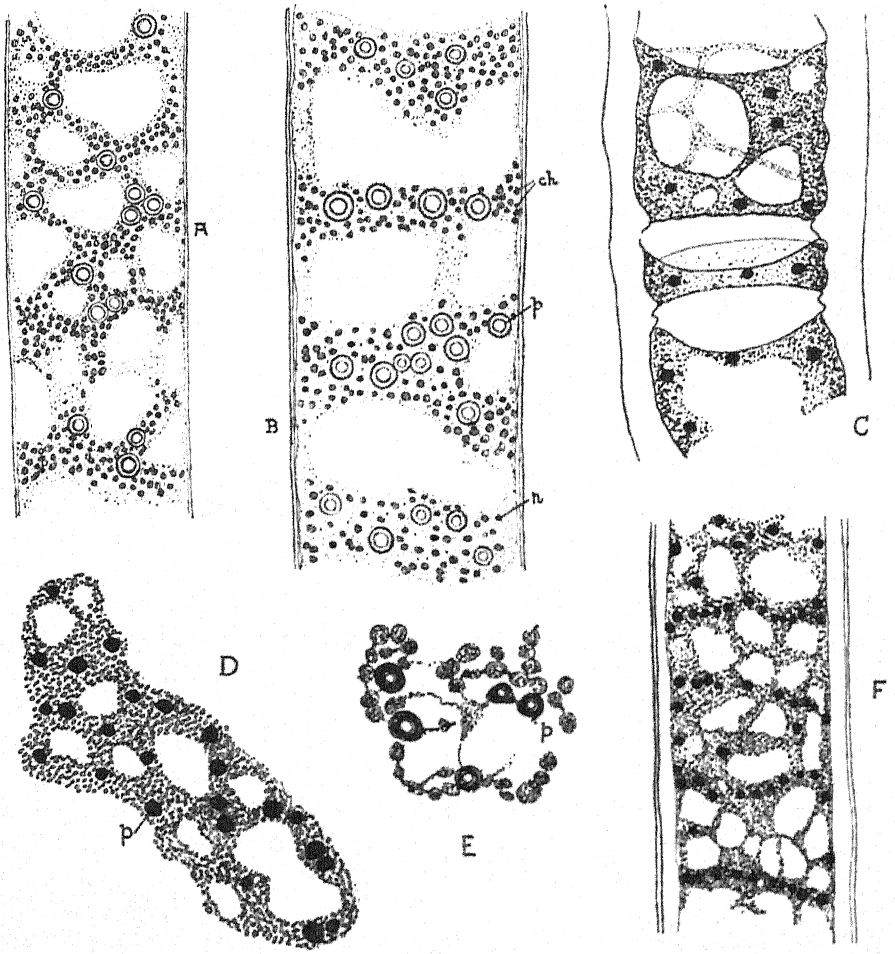


FIG. 3. Chloroplasts of *S. africana*, Fritsch (A, B), *S. annulina* (Roth), Ag. (C), and *S. africana* var. *crassa* nov. var. (D–F). C shows the reticulation of the chloroplasts of *S. annulina*, E the fragmentation of the chloroplast of *S. africana* var. *crassa*. *ch.*, starch-grains; *n.*, nucleus; *p.*, pyrenoids. (A and B × 520; C × 775; D × 400; E × 550; F × 185).

tend to occur especially in fertile material (cf. Fresenius 4, t. vi, Figs. 21, 23; Rauwenhoff 17, pp. 99, 126, t. iii, Fig. 5).[1] The condition of the chloroplasts figured by G. S. West (21, p. 274, Fig. 176 A) would also seem to be of this nature, but I have not encountered anything exactly like this.

[1] 'Il en résulte que le contenu de la cellule, bien que toujours d'un vert vif, prend un autre aspect général, les anneaux étant en grande partie remplacés par un réseau à larges mailles' (Rauwenhoff, p. 126, Development of oogonia).

By contrast to the other species *S. africana* has its chlorophyll disposed in a diffuse network, not unlike that found in the cells of *Cladophora* (Fig. 3 A, D). In places the network is dense, at other points there are wide and more or less irregular meshes (Fig. 3 F). Relatively large pyrenoids, with a well-marked starch-sheath, are irregularly scattered in considerable numbers, while in the intervening matrix there are often numerous small elliptical starch-grains. In the type one may find at rare intervals a disposition of the chlorophyll in transverse rings (Fig. 3 B) and, since only fertile material has been examined, it is not impossible that in the vegetative stage this annular arrangement may be more widely represented. But it must be realized that in *S. annulina*, in the same stage of reproductive activity, typical annular chloroplasts are abundantly found. In the var. *crassa*, although I have had the opportunity of examining material collected on a number of separate occasions, no clear signs of an annular arrangement have been noticed; at the best, one finds in an occasional segment indications of a grouping of pyrenoids in transverse series (Fig. 3 F). It seems probable that here, even in the vegetative phase, the chlorophyll is always disposed in a diffuse network, and the same is not unlikely to be true also of the type. An investigation of the young filaments of these two forms will be of interest.

It is probable that, in these forms with a diffuse reticulate chloroplast, fragmentation of the latter is a frequent, if not regular phenomenon. I am indebted to Dr. N. Carter for the opportunity of examining stained preparations showing this feature (cf. also Fritsch 6, p. 526). Subsequently Dr. E. M. Delf very kindly supplied me with beautifully-fixed material of var. *crassa*, collected during her stay in South Africa, and this showed the feature in question quite clearly without staining (Fig. 3 E). There can be no doubt, however, that this structure arises from an originally entire reticulum as a secondary modification.

The typical arrangement of the chlorophyll in *S. tenuis* is in transverse bands which here, in conformity with the narrowness of the threads, usually harbour only one or two pyrenoids (Fig. 4 A, B); when single, the latter are often very large, exceeding half the width of the thread. Longitudinal connexions between adjacent bands are frequent (Fig. 4 B), and are probably indicative of recent division. It has not been possible to settle the exact shape of these chloroplasts which are sometimes longer than broad; it seems probable that the chloroplast, occupies most, if not the whole, of the width of the thread. In a considerable number of the filaments a much more diffuse distribution of the chlorophyll occurred (Fig. 4 O); this is possibly a stage in the development of reproductive cells, and Fig. 4 C may represent an intermediate condition.

In the forms with annular chloroplasts the nuclei are practically confined to the rings (Heinricher 11, p. 438; Klebahn 12, pp. 86, 94), the number in each ring varying considerably; in *S. annulina* var. *crassiscpta*,

according to Klebahn and Heinricher, there are only one to four, most commonly two. Dr. Carter informs me that nuclei are only very exceptionally found between the chloroplasts. As pointed out by Klebahn (loc. cit., p. 86), the nuclei are relatively large, compared with those of other coenocytic Algae.

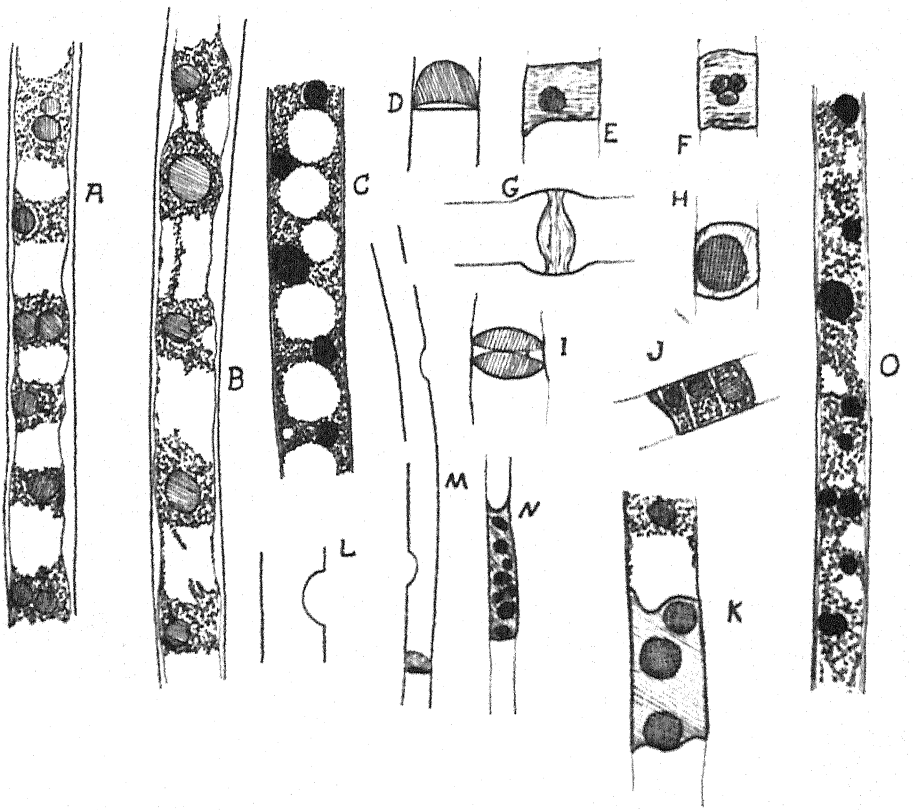


FIG. 4. *S. tenuis* n. sp. A and B, normal vegetative threads (pyrenoids shaded). C and O, threads preparing to form reproductive cells (pyrenoids black). D, G, I, septa, the shaded portions in D and I represent the deposit mentioned in the text. E, F, H, J, K, N, the 'plugs' (pyrenoids shaded). M, segment which has liberated its gametes, showing the numerous large pores. L, one of the latter enlarged. (A–E, H, I, K, L × 880; F, J, and O × 703; M, N × 345).

In *S. africana* and its var. *crassa*, on the other hand, the nuclei are quite irregularly scattered and smaller than those of *S. annulina*. I am again indebted to Dr. Carter for this information. The nuclei of *S. tenuis* are very small and appear to show no regular distribution.

We have thus within the genus *Sphaeroplea* a distribution of the cell-contents of two types. In *S. annulina* and *S. zeilmani* there are separate annular chloroplasts and the nuclei, often relatively few, are practically confined to the region of the chloroplasts.

The chlorophyll-apparatus of *S. tenuis* is essentially of the same type.

On the contrary, in *S. africana* (incl. var. *crassa*) we have a diffuse reticulate chloroplast and numerous scattered nuclei. It is probable that in certain forms of *S. annulina* a more or less marked reticulation of the annular chloroplasts may occur, and that in *S. africana* some traces of an annular arrangement may be found. It can hardly be doubted that the condition in *S. africana* is an advance on that of the other species, and it is possibly to be related to the relatively greater width of the threads of this species. It remains to be seen whether the young stages of *S. africana* show an annular disposition of the chlorophyll, from which the diffuse reticulum originates. Unfortunately, the chlorophyll-apparatus of *S. cambrica* is at present completely unknown.

(*c*) The Reproductive Organs.

Sphaeroplea is the only oogamous green alga in which the vegetative segments constitute the reproductive organs without any change of form, and in this respect it is not more highly differentiated than the majority of isogamous types. It is not proposed to deal here with the details of development of the ova and spermatozoids (cf. Klebahn 12). The oogonia occur singly or in short series.[1] As a general rule the entire protoplast is used up in the formation of the ova, but in *S. africana* a small shrunken mass occasionally remains. According to Klebahn (p. 88), there is no nuclear division during the development of the ova. The latter are usually uninucleate, although Klebahn (loc. cit., p. 88 ; cf. also Meyer 14, p. 66) records multinucleate ova (with as many as six nuclei) as common in the form of *S. annulina* known as var. *braunii*; there are always several pyrenoids. The spermatozoids in *S. annulina* contain a single nucleus and abundant nuclear division takes place in the antheridium prior to their development (Klebahn, p. 86) ; it is noticeable, that, by contrast to the oogonia, the annular chloroplasts remain recognizable in the antheridia until a late stage.

Very numerous ova and oospores are produced within the oogonia. They often lie in a single series in *S. wilmani* (Fig. 5 C), in from one to three irregular rows in the various forms of *S. annulina*, as well as in *S. cambrica*, while in *S. africana* (Fig. 5 A), and especially in its var. *crassa* the numbers are so great that no definite arrangement is obvious. In all species the oospores are frequently so closely packed that practically no space remains within the oogonium, and, in such cases, they often become distorted as a result of mutual pressure. Sometimes the oogonium-membrane is irregularly bulged out at certain points by massing of the contents. In proportion to the width of the oogonia, the ova and oospores of *S. africana* are relatively smaller than those of the other species. Abnormally large

[1] In the following account *S. tenuis* is ignored, its reproduction being described separately towards the end of this section (p. 18).

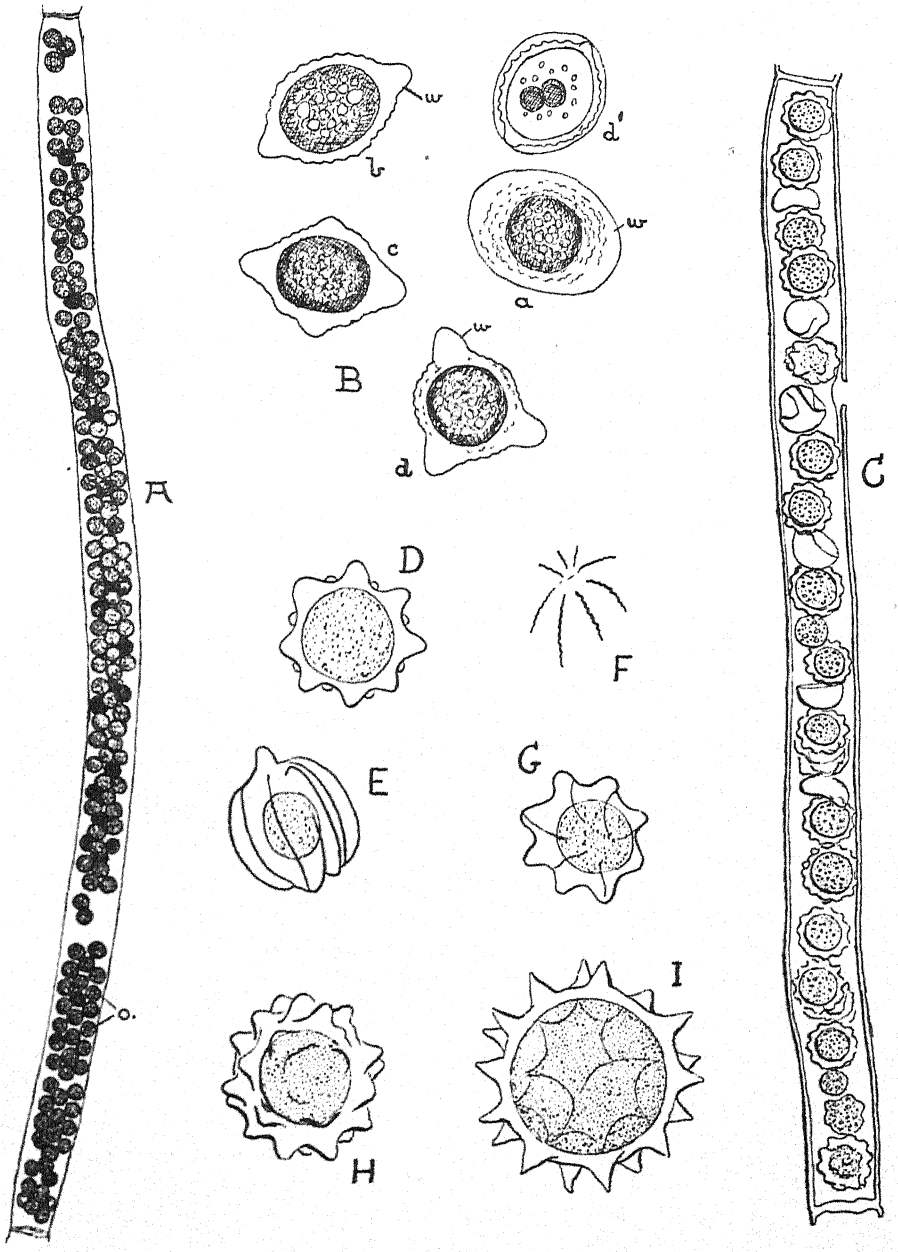


FIG. 5. A and B, *S. africana*, Fritsch; A, oogonium; B, oospores (see text). C, *S. wilmani*, Fritsch and Rich, oogonium with almost mature oospores. D–G, *S. cambrica* n. sp., oospores; E, side-view; D and G, end-views (in G slightly oblique). H, *S. wilmani*, oospore. I, *S. annulina* (Roth), Ag., oospore. (A × 140; B × 675; C × 420; D–G × 560; H × 850; I × 990).

spores are occasionally met with in most species, and have frequently been recorded in *S. annulina*.

The apertures, through which the spermatozoids penetrate, are not numerous and appear to be quite irregularly distributed. As a general rule they are round, but in *S. africana* they often show an irregular edge, and commonly at least are wider in the transverse than in the longitudinal direction (Fig. 6 A). It would be of interest to establish their exact location in the species possessing annular chloroplasts, a matter that should not be difficult in the case of the antheridia. It would not be surprising if one aperture were found to correspond to each ring.

The structure of the oospores is the most variable feature within the genus, and it is upon this character that the specific distinctions are mainly to be based. In all cases the fertilized ovum becomes enveloped by a close-fitting, thin, smooth hyaline membrane, and it is within this that the permanent membranes are gradually laid down (Fig. 5 B d′; Fig. 6 E, J; Fig. 7 E, F; cf. also Fresenius 4, p. 245; Rauwenhoff 18, p. 101; Fritsch 6, p. 530). As a general rule, one can find within the same oogonium oospores that are still enclosed within the primary membrane, as well as others that have shed it; in the latter case the empty membranes are found lying alongside of the mature oospores (Fig. 5 C). It appears to be essentially distinctive of the South African *S. wilmani* that a number of primary membranes are formed which are shed successively, so that several empty membranes may be found corresponding to each mature oospore.

There are two main forms of oospores occurring in *Sphaeroplea*, the spherical types provided with uniformly distributed spiny (Fig. 5 I) or blunt wart-like (Fig. 5 H) outgrowths, and the types provided with a number of conspicuous wing-like ridges in which one axis is commonly longer than the other (Fig. 5 B and E). The spherical type is characteristic of *S. annulina* and *S. wilmani*. In the former the outermost membrane of the mature oospore bears evenly distributed short, usually hollow spines, more or less conical in form but with blunt apices (Fig. 5 I, 6 G); they would appear usually not to be very numerous, but in some of the South African specimens of *S. annulina* the spines on the oospores are very closely set (Fig. 6 H and J). The bases of the spines are united by a regular network of delicate ridges which form a polygonal pattern, the meshes commonly being pentagonal (Fig. 5 I, 6 G, H′, J). These very characteristic spores are well figured by Heinricher (11, t. xii, Fig. 12), G. S. West (21, p. 274, Fig. 176 C), and Lowe (13, t. ii, Fig. 16). Close packing of the oospores (cf. above) may lead to almost square or semi-tetrahedral forms, but these are quite abnormal and scantily represented. Occasional ones are ellipsoidal.

In *S. wilmani* the processes are always relatively few, solid, and broader and blunter than those of the oospores of *S. annulina* (Fig. 5 H; 6 K), having more the character of warts than spines. Moreover, the ridges on the mem-

brane are quite irregular (Fig. 6 L), sometimes running almost parallel to one another, more usually following a devious course; they do not by any means

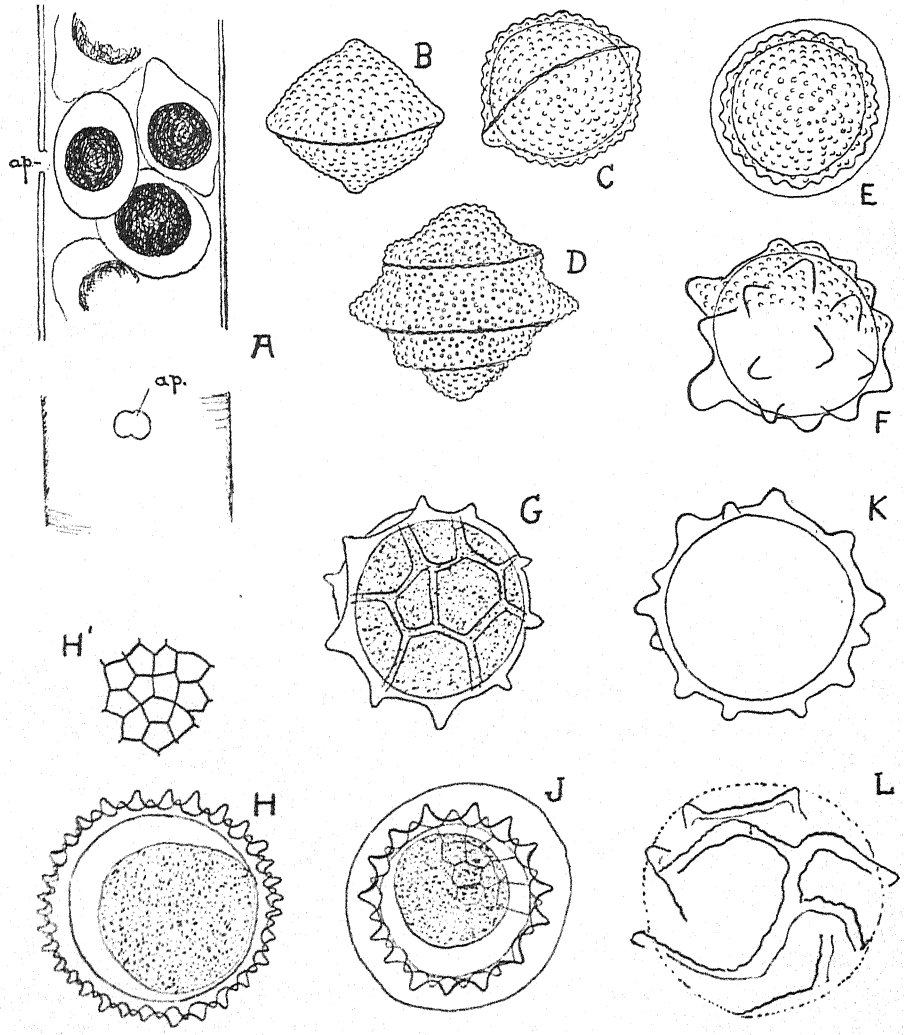


FIG. 6. A, *S. africana*, Fritsch, small part of oogonium with young oospores. B–F, *S. africana*, var. *crassa* nov. var., oospores; E, young oospore; F, abnormal form. G, *S. annulina* (Roth), Ag., oospore. H–J, *S. annulina* forma *polyspinosa*, oospores; H, in optical section; H', small part of the network of ridges seen from the surface. K, L, *S. wilmani*, Fritsch and Rich, oospore; K, in optical section; L, the same seen from the surface. *ap.*, aperture of oogonium (A × 630; B–F × 980; G–J × 1,075; K, L × 1,280).

always connect with the processes and commonly seem to be quite independent of them. They are much more strongly developed than in the other species, and often partake of the character of wings. Frequently the membrane between the ridges is finely granulate, which may give a crenulate

appearance in certain aspects. The oospores of this species are always completely spherical.[1]

The spores of *S. africana* are of quite a different type. They are broader than long (Fig. 5 B, c ; 6 B) and usually appear elliptical in side-view (Fig. 5 B, b), although often approximately circular in end-view (Fig. 5 B, a). They are provided with a distinct median wing (Fig. 5 B, a, b, w) which can be traced all the way round the equator of the spore (Fig. 6 B, C), while the poles are often slightly produced into crests (Fig. 5 B, c ; 6 B). Side by side with this, which is by far the commonest type, one finds occasional tricarinate spores provided with three blunt processes (Fig. 5 B, d). In the var. *crassa*, while Fig. 6 B depicts the common type, greater variability was found. Thus the polar crests were sometimes lacking (Fig. 6 C) or even the median wing (Fig. 6 E, which may, however, be an immature stage) ; on the other hand, Fig. 6 D shows a spore in which two accessory wings are developed parallel with the usual one. A very exceptional and abnormal type is seen in Fig. 6 F, where numerous blunt processes are developed, and the normal shape is altogether lost ; such a spore is very similar, except for the absence of ridges, to that of *S. wilmani*. In all cases the membrane is beset with numerous rather coarse granulations which impart a fine crenate character to the margins.

The most elaborate oospores are found in *S. cambrica*. The spores are here nearly always longer than broad (Fig. 5 E ; 7 E, F), although isodiametric in cross-section (Fig. 5 D ; 7 A). The membrane is traversed by eight finely crenulate wings, running approximately parallel with or frequently a little oblique to the long axis of the spore (Fig. 5 E ; 7 E) and appearing as blunt protrusions in end-view (Fig. 5 D ; 7 A). Two of these eight ribs are median, and would appear usually to form one structure, being continuous over the poles,[2] whilst the other six are more or less symmetrically disposed on either side of this median line, but do not extend right up to the ends, terminating just short of them (cf. Fig. 5 F ; 7 A, C); these latter ribs seem often to be produced into a papilla at one or both (?) ends (Fig. 7 B, F), but this appears to be a variable feature, and may in part be exaggerated by the spores often lying in somewhat oblique positions. Not uncommonly the ends of some of the ribs appear to be connected across the face of the spore by slight irregular ridges, but this does not always seem to be the case (cf. Fig. 7 E, F, H). The surfaces between the ribs are concave (Fig. 7 A), and are marked by fine undulating lines along at least a part of their length (Fig. 7 C, D, H). Not uncommonly there are smaller secondary ribs between the eight principal ones (Fig. 5 D ; 7 D) ; these usually extend only for a short

[1] The description of the oospores given by Fresenius (4, pp. 245, 246) recalls to some extent the characters of *S. wilmani*, and it is not altogether out of the question that he may have been dealing with that species (cf. also Hansgirg, 9, p. 52).

[2] It is not certain, however, that the same pair of ribs is continuous at the two opposite poles.

distance in the middle region of the spore, and are too short and faint to be seen in end-view, but sometimes they are more pronounced, and, in the exceptionally large oospores that are sometimes found, they may be so

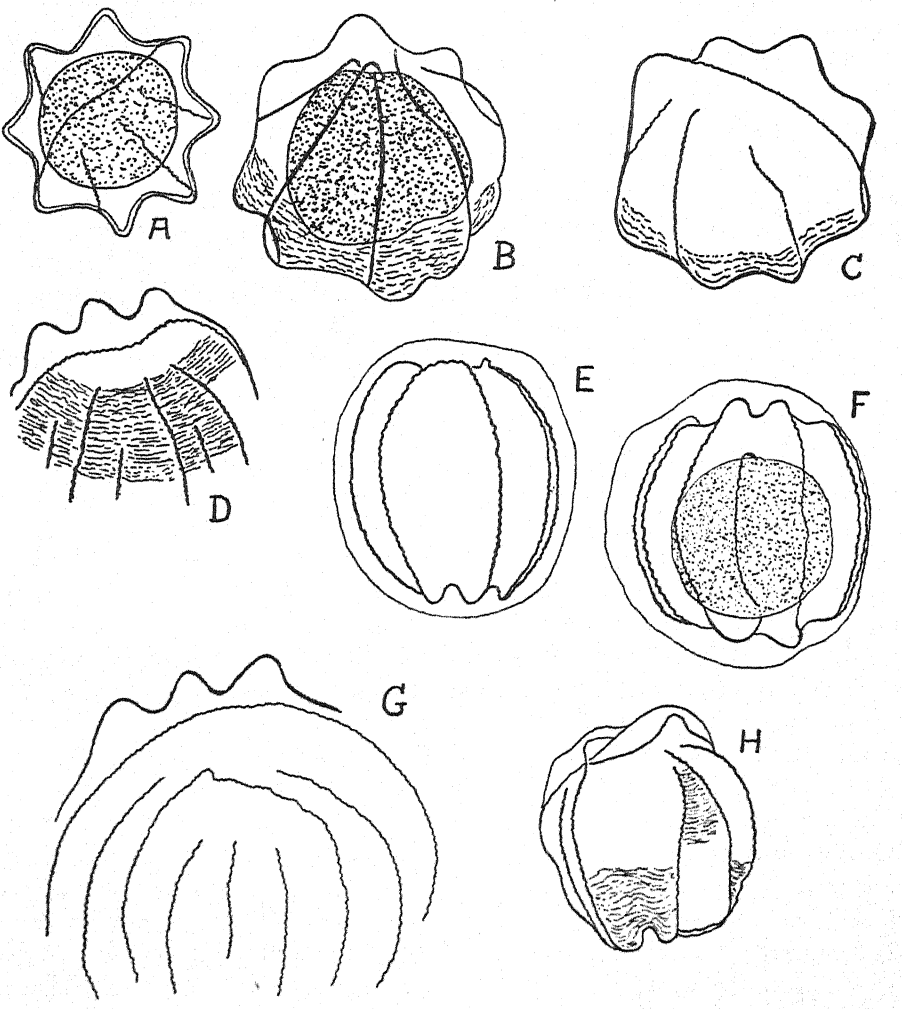


FIG. 7. *S. cambrica* n. sp., oospores. A, end-view; B–D and G, oblique end-views. E, F and H, from the side. G is an exceptionally large oospore with sixteen wings. (All figs. × 900).

marked that the oospores possess sixteen nearly equal ribs (Fig. 7 G. In some of the oogonia of *S. cambrica*, with a single row of oospores, the latter were placed with the long axis transverse to the filament over long stretches.

The close packing of the spores, to which repeated reference has already been made, would seem to imply an appreciable enlargement after fertilization. The distortion, which results from it, is responsible for the somewhat

marked degree of polymorphism exhibited by the oospores, as a consequence of which forms are sometimes produced somewhat resembling those typical of another species. The fact remains, however, that, in the four types described, the majority of the spores are perfectly constant in their characteristics and readily distinguishable from one another.

It remains to consider the reproduction of *S. tenuis*. In the material of this species there occurred an abundance of filaments with empty segments provided with numerous, and usually remarkably large apertures (diam. 8–12 μ) with a circular opening (Fig. 4 L, M). In spite of diligent search on several occasions, however, no traces of oogonia were found, although many of the threads showed an arrangement of the contents that might well represent the preliminary stages in the formation of reproductive cells (Fig. 4 C and O). The conclusion seems justified that in this species the latter are liberated to the exterior before fertilization.

Among the tangle of threads, and especially on the surface of fragments of a *Phormidium*-stratum, there were found numerous spores, only slightly wider (usually 14–15 μ without the spines) than the average diameter of the threads of *S. tenuis*, with a thick membrane beset with numerous pointed spines (Fig. 8 A–D). Apart from the absence of ridges between the spines, these spores much resembled the oospores of *S. annulina*; as a general rule, they contained two pyrenoids (Fig. 8 D). This type of spore is, however, of such wide distribution among the Algae that without further evidence one would not be inclined to attribute it to *S. tenuis*, in spite of the similarity of dimensions. On closer investigation, however, it was found that many of these spores were enclosed in a rather delicate smooth hyaline membrane (Fig. 8 B), while others were found lying just next to a membrane which had obviously been shed (Fig. 8 C and D). This indicates a method of development of these spores, which is characteristic of the oospores of *Sphaeroplea*, and constitutes considerable presumptive evidence that they belong to *S. tenuis*. There were also present many rounded or somewhat elliptical cells of the same dimensions (12–15 μ), but provided with a smooth membrane (Fig. 8 E). These may represent younger stages, but since no intermediate conditions between these and the stage shown in Fig. 8 B were found, and since, moreover, there were present in the same material threads of *Ulothrix* showing abundant aplanospores of about the same form and dimensions, this interpretation cannot be certified.

Some of the frequent fragments of empty segments of *S. tenuis* contained occasional elliptical structures with two pyrenoids and enclosed in a thin smooth membrane (Fig. 8 F). In a few cases elliptical spores, with the same spiny envelope as the ordinary spherical ones, were found lying just outside an empty membrane of this type, the latter within a fragment of a *Sphaeroplea*-segment (Fig. 8 G). It can hardly be doubted that these elliptical spores belong to *S. tenuis*, and the facts just detailed lend support to

the view that the kind of structure shown in Fig. 8 E is an early stage in the development of the typical spherical spore.

There is thus good evidence that in *S. tenuis* both types of gametes are liberated to the exterior, the pores being quite sufficiently wide to allow of the escape of swarmers, not much smaller than the spores. It is possible that, hand in hand with this more primitive condition, there may go a less

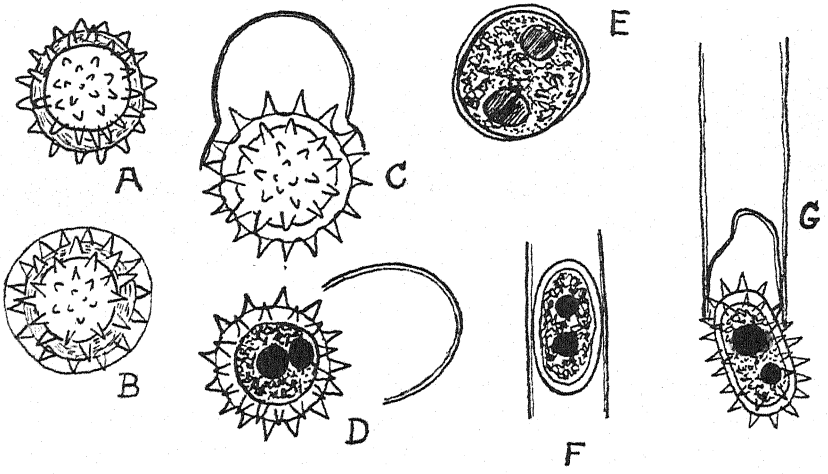


FIG. 8. *S. tenuis* n. sp., probable oospores or zygospores and stages in their formation, see p. 18. B shows the membrane surrounding the young spore; in C and D it has been shed; E and F, possible young stages; G, elliptical spore. (All × 1,100).

extreme differentiation of the gametes. *S. tenuis* may not even be oogamous! Stages in the fusion of two slightly unequal gametes were not uncommonly encountered in the sediment of the sample containing *S. tenuis*, but these may of course have belonged to another alga, although the dimensions were suitable. The elliptical spores above mentioned might have arisen by the fusion of two gametes, one of which had failed to escape from the *Sphaeroplea*-segment. Within the close confines of the narrow thread the zygote would necessarily assume an elliptical shape.

The germination of the oospores is known only in *S. annulina*. As a general rule, the contents divide to form four biciliate zoospores (Heinricher 11, p. 444; Rauwenhoff 18, p. 107; Meyer 14, p. 70), although two, one, or eight are sometimes produced (cf., however, Meyer 14, p. 75). According to Cohn (3, p. 194; also Meyer 14, p. 77), these zoospores develop a membrane during their period of movement. As will be familiar, they come to rest without reaching a substratum, so that the threads are free-floating from the beginning. Many workers have described the characteristic spindle-shaped filaments that first arise from the lengthening body of the zoospore. Heinricher (p. 449) records that such young plants may reach a length of $3\frac{1}{2}$ mm. without a septum being produced.

Sphaeroplea is unique among the Green Algae in the fact that zoospores are formed from the germinating oospores, but are not known to be produced by the ordinary vegetative threads. There are unrivalled opportunities in South Africa for an investigation of this matter, which is sufficiently striking to require careful confirmation.

(*d*) The Affinities of *Sphaeroplea*.[1]

Sphaeroplea is the only oogamous green alga of which the oogonia and antheridia are constituted by the ordinary vegetative segments. It also stands alone among Isokontae in the fact that numerous (often 100 or more) ova are formed in the oogonium. In both respects it shows very primitive characters, and, from these points of view, its sexual organs are directly comparable with those of Cladophoraceae or of *Ulothrix*, whereas in Siphonales there are usually specially-differentiated gametangia. The method of release of the male cells and of their admission to the female, viz. by the solution of small parts of the membrane of the gametangium to form more or less rounded apertures, is very analogous to that obtaining in the forms just mentioned, with this difference, that in an *Ulothrix*, &c., only one aperture is formed, whereas in *Sphaeroplea* there are many. The state of affairs in *S. tenuis*, where evidently neither gamete is retained, shows a still more primitive condition and, as above indicated, it is quite possible that *S. tenuis* is not even oogamous, while it is highly probable that both gametes are motile.

In *S. annulina*, *S. tenuis*, and *S. wilmani* the segments normally contain a number of separate chloroplasts which, in the first and last species, take the form of a ring with a smooth or slightly-toothed edge, whilst in *S. tenuis* they are much narrower, and probably occupy most of the width of the segment. The annular chloroplasts are exceedingly similar to those found in many forms of the species-aggregate *Ulothrix zonata*, whilst those of *S. tenuis* are not unlike the chloroplasts of some of the smaller species of *Ulothrix*. If in such a form of *Ulothrix zonata* no septa were to be produced over long stretches, we should obtain a condition practically identical with that found in *S. annulina* or *S. wilmani*. It is further to be noted that in these species the nuclei are practically confined to the regions of the chloroplasts, that there is sometimes only one nucleus to each ring, and that the pyrenoids vary in number in accordance with the widths of the threads, being commonly single in the chloroplasts of *S. tenuis*. It is also well to recall Gutwinski's record mentioned on p. 18 of a form of *S. annulina* possessing quite short 'cells' with only one or two chloroplasts. The facts point to the interpretation of each chloroplast with its associated nuclei in

[1] The main substance of this section was given before section K at the Leeds meeting of the British Association.

these species of *Sphaeroplea* as a single entity, the whole segment consisting of a series of such entities. And each of them may be directly compared with a single *Ulothrix*-cell. It is clear, however, that in *S. africana* and its var. *crassa*, the separate chlorophyll-bands of the other species are replaced by a diffuse reticulate chloroplast, analogous to that found in a *Cladophora*, and that hand in hand with this goes an irregular distribution of the nuclei. Intermediate stages are sometimes found in *S. annulina* (Fig. 3 C) and in the ordinary form of *S. africana* (Fig. 3 B).

The view that *Sphaeroplea* is probably derived from a septate Ulotrichaceous type, which has ceased to form septa except at rare intervals,—an advance which has gone hand in hand with the acquisition of a primitive type of oogamy,—receives some further support from the fact that septation is apparently tending to disappear altogether in some species of the genus. In *S. tenuis* normal septa are few and far between, while in *S. africana* and the var. *crassa* many of the peculiar septa are quite incomplete and probably merely mechanical contrivances rather than true cross-walls. It is to be noted that this feature in *S. africana* is associated with a more advanced type of chlorophyll-apparatus and with more elaborate oospores.

In one other respect *Sphaeroplea* shows a clear affinity with the Ulotrichales rather than with the Siphonales. All accounts agree that the oospores of *S. annulina* (the only form studied from this point of view) produce a number of zoospores in germination. This is as in *Ulothrix*, whereas the zygotes of *Codium*, *Bryopsis*, *Vaucheria*, &c., all germinate direct.

It is clear that a consideration of all the facts removes *Sphaeroplea* from the Siphonales, and brings it into rather close relation with the Ulotrichaceae. Upon this point there would never have been any misconception, except for the siphoneous character of the alga, but that is seen to be so clearly derivable from the *Ulothrix*-type of construction that it would be a mistake to attribute undue importance to it in face of all the facts that speak the other way.

(*e*) Taxonomy.

As already stated above, the taxonomy of the species of *Sphaeroplea* must be based essentially on the structure of the oospores, although features of the cell-contents are also of value. At present five species of the genus may be distinguished, three of which are as yet only known to occur in South Africa, whilst one is only known from Wales. The following is a key for their determination :

(*a*) Oospores spherical, their membrane provided with spines or blunt warts; chloroplasts forming successive transverse rings or bands, the nuclei probably restricted (always?) to the region of the chloroplasts.

(1) Oospores not formed within the oogonium, both kinds of gametes liberated

before fertilization, threads very narrow (not exceeding 15 μ) with chloroplasts usually in the shape of transverse bands with one or two pyrenoids—*S. tenuis.*

(2) Oospores formed within the oogonium, chloroplasts usually annular with a number of pyrenoids, threads generally much wider than 15 μ.

(i) Oospores provided with blunt conical spines whose bases are connected by a regular network of delicate ridges forming a polygonal pattern—*S. annulina.*

(ii) Oospores provided with broad and blunt warts, between which is a system of coarse wing-like ridges, not regularly connecting the bases of the warts—*S. wilmani.*

(*b*) Oospores not spherical, one axis longer than the other, their membrane provided with wing-like ridges which are symmetrically disposed, chloroplasts (not known in *S. cambrica*) in the form of an irregular reticulum, nuclei scattered.

(1) Septa complex and built up of a number of separate finger-like processes, oospores with a median wing, sometimes tricarinate, the membrane coarsely granulate—*S. africana.*

(2) Septa not composed of separate processes, oospores elliptical, typically with eight longitudinal wing-like ridges which are symmetrically disposed—*S. cambrica.*

It is not improbable that, when further details of *S. tenuis* are known, it will be found advisable to establish a separate genus for this remarkable form. Similarly, the rather exceptional type of cell-contents and septa of *S. africana* may warrant generic separation also for this species. The writer has felt, however, that further study of these two forms on fresh material is desirable before deciding upon the merits of such a step. In view of such possibilities no amended diagnosis of *Sphaeroplea* is given here, but this will manifestly be necessary, if these two species are retained within the genus.

The following are brief diagnoses of the five species giving only their essential characters:

(1) *S. annulina* (Roth), C. A. Agardh, Syst. Alg., 1824, p. xxv (Syn.: *Conferva annulina*, Roth, Catalecta bot., iii, 1806, p. 211, tab. VII; *S. trevirani*, Kütz., and *S. braunii*, Kützing, Spec. Alg., 1849, p. 362) (Fig. 2 B–D, F, G; 3 C; 5 I; 6 G). Threads 24–72 μ broad, each segment containing a considerable number of annular chloroplasts with 2–10 pyrenoids; septa simple, thin, or in certain forms strongly thickened and complex, but not clearly constructed of separate processes. Oospores in one or more series, spherical, with a thick membrane bearing somewhat blunt conical hollow spines, whose bases are connected by a regular polygonal network of delicate ridges, often with pentagonal meshes. Diam. oospor., 11–36 μ (most usually 11–20 μ).

This species is known from all continents except Australia, and detailed

descriptions of it will be found in most algal floras. It is probable, however, that it has occasionally been confused with other species, and stress may once more be laid on the chloroplasts and oospores which clearly characterize it. Printz (17, p. 298) speaks of *S. annulina* as very variable, but I can find no evidence that it varies in these two essential respects and, as above indicated (p. 14), excellent figures of the typical oospores have been published from various parts of the world. Kützing (loc. cit.) adopted the name of *S. trevirani* for the form originally described by Roth, and, apart from this, distinguishes three other species, one of which (*S. braunii*) has been substituted by various subsequent writers for the name *annulina*; but the latter clearly has priority. Kützing's four species differ from one another only in the width of the threads and the number of rows of oospores. Rabenhorst (Flor. Europ. Alg., iii, 1868, p. 318) places them all as synonyms of *S. annulina*, but Hansgirg (9, p. 52), Kirchner (Algenfl. v. Schlesien, 1878, p. 64), and De Toni (Sylloge Algarum, i, 1889, p. 95) adopt them as varieties of this species. Such differences are, however, worthless, since there is considerable variation in these respects within the same mass of material (cf. Meyer 14, p. 80).

Heinricher's var. *crassisepta* has been adopted as a separate species by some (e. g., (10), p. 65; Migula, Kryptogamenflora, ii, 1, 1907, p. 860), whilst others regard it only as of varietal value. Collins (Green Algae of North America, Tufts Coll. Stud., ii, No. 3, 1909, p. 385) states that all American specimens are of the *crassisepta* type, but Lowe (13) seems to have had the form with thin septa. It would seem that the curious septa on which var. *crassisepta* is essentially based are somewhat variable in their occurrence (cf. especially also Meyer 14, p. 82). It will be convenient, however, to retain a var. *crassisepta* for types of *S. annulina* in which such thick complex septa are the rule; whether the other characters (size of cell, number of pyrenoids in the chloroplasts, &c.), on which Klebahn (12, p. 98) based his claim for specific separation, always go hand in hand with the thick septa may be doubted. In the material of *S. annulina* from Griqualand West the oospores bear the spines more closely set than is usual in this species; this form may be distinguished as forma *polyspinosa* (Fig. 6 H, J). No other forms or varieties can be recognized.

(2) *S. wilmani*, Fritsch and Rich, 7, (Fig. 2 H–L; 5 C, H; 6 K, L). Threads 24–34 μ broad, each segment containing many annular chloroplasts often rather more delicate than those of *S. annulina*; septa thick and coarse. Oospores commonly in a single series, spherical, developing a number of primary membranes which are shed successively, the mature membrane thick and provided with a few broad and blunt solid processes, and a system of coarse irregular ridges that show no definite relation to the processes. Diam. oospor., 23–27 μ Monoecious.

This species is at present known from the Hart's River and from

a stream at Hay, Griqualand West, also from the neighbourhood of Cape Town, and there are some indications that a similar form has been found in Europe (cf. footnote on p. 16).

(3) *S. tenuis* n. sp. (Figs. 4 and 8). Threads 8·5–15 μ (usually 10–12 μ), broad, segments usually very long, chloroplasts in the shape of numerous transverse bands with only one or two pyrenoids, rarely more diffuse; septa few, usually thin, but commonly covered with an opaque whitish deposit that also occurs as an occlusion at frequent points in the threads, these occlusions including one or more (sometimes many) pyrenoids (see p. 6). Gametes liberated to the exterior prior to fertilization, the holes in the walls of the gametangia being large (diam. 8–12 μ) and rather numerous. Oospores (?, cf. p. 18) spherical with a thick membrane bearing closely-set spines not connected by ridges, and formed, as in other species, within a smooth hyaline primary membrane, which is subsequently shed. Diam. spor., 12–15 μ (usually 14–15 μ). The assignation of these spores to *S. tenuis*, while probably correct, requires confirmation.

This species was collected in December, 1927 by Mr. J. H. Power from a spring at Niekerk's Hoop, Hay, Griqualand West.

(4) *S. africana*, Fritsch, 6, p. 524 (Fig. 1 A–I; 2 A, E; 3 A, B; 5 A, B; 6 A). Threads 42–78 μ, broad in the middle of the segments (19–54 μ at the septa), segments barrel-shaped, chlorophyll normally arranged in a diffuse reticulum with many large scattered pyrenoids, occasionally in ill-defined transverse rings with an irregular row of pyrenoids; septa complex, composed of a large number of separate finger-like processes growing inwards from the periphery and often failing to reach the centre, so that many of the septa are incomplete. Oospores broader than long, with a distinct median wing, rarely tricarinate, membrane coarsely granulate. Dimens. oospor., 20 × 12, 27 × 15, 27 × 18, 33 × 21 μ.

This species is known from temporary 'vleis' in the neighbourhood of Cape Town. Another Cape Town form is

Var. *crassa* nov. var. (Figs. 1 J–L; 3 D–F; 6 B–D). Threads 112–170 μ broad, chloroplast always a diffuse reticulum, probably usually composed of many more or less distinct pieces; septa very thick (up to 34 μ thick) and complex, but constructed as in the type. Oospores as in the type. Dimens. oospor., 21 × 26, 22 × 29, 22 × 31, 26 × 29 μ.

(5) *S. cambrica*, n. sp. (Figs. 5 D–G; 7). Threads 34–51 μ broad, segments up to 1700 μ in length, but often shorter, chloroplasts unknown; septa relatively thin and simple. Oospores closely packed, ellipsoidal, provided with eight prominent wings running longitudinally from pole to pole, two connected over the summit, the others stopping just short of it, and often terminating in slight papillae. Dimens. oospor., 27 × 29, 29 × 31, 32 × 34, 38 × 43, 46 × 51, 51 × 54 μ.

This interesting species was found by Professor Lloyd Williams in

pools between sand-hills at Morfa Bychan, S. Carnarvonshire. It was recorded as *S. annulina* by the writer (22, p. 173) before he was aware that material was available for examination. Unfortunately this material consists solely of oogonia densely packed with the peculiar oospores.

(*f*) Summary.

The paper deals with the morphology, affinities, and taxonomy of the genus *Sphaeroplea*, the account being based on the five species, *S. africana*, *S. annulina*, *S. cambrica*, *S. tenuis*, and *S. wilmani*. While most species possess more or less normal septa, those of *S. africana* are invariably composed of a series of radial finger-like processes which, moreover, commonly fail to meet in the centre, so that no true septum is produced. In *S. annulina* and *S. wilmani* the chloroplasts constitute transverse rings, in *S. tenuis* transverse bands, but in *S. africana* their place is taken by a diffuse reticulum with scattered pyrenoids. It is suggested that within the genus one can trace a tendency towards loss of septation, and towards the development of a diffuse reticulate chloroplast, from the primitive type illustrated by *S. annulina* or *S. wilmani*. This view receives confirmation from the fact that in the suggested advanced types the oospores are more elaborate than in the others, being elliptical and provided with wings in contrast to the spherical type of oospore found in the simpler types. *S. tenuis* is quite exceptional in the fact that both kinds of gametes are evidently liberated prior to fertilization, and that the oospores (?) are formed in the outside medium. In the section dealing with the relationships of *Sphaeroplea* it is emphasized that all the evidence indicates an affinity with Ulotrichaceae rather than with Siphonales, and it is pointed out that *Sphaeroplea* can be derived without difficulty from a simple *Ulothrix*-like form. Further study may warrant the establishment of separate genera for *S. africana* and *S. tenuis*.

I am indebted to my colleague Dr. N. Carter for valuable suggestions with reference to the detailed cell-structure; to Miss E. L. Stephens, Dr. E. M. Delf, Professor W. Robinson, and Mr. A. D. Cotton for the supply of material; and to Miss F. Rich and Dr. E. J. Salisbury for help in the preparation of the figures.

East London College,
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The Relation of Nitrogen Metabolism to Plant Succulence.

BY

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FOR some time it has been a matter of common experience among plant physiologists and horticulturists that plants receiving abundant supplies of nitrogenous fertilizers generally possess unusually high water content, together with a general appearance of succulence. For this reason soils exceptionally rich in nitrogen are not always regarded favourably in horticultural practice, because the crops grown on them, although exceptionally heavy, are difficult to handle and market on account of their succulence. In spite of the common knowledge of this phenomenon, there seems to be little or no evideuce as to its nature, and consequently the following pages are offered as some contribution towards the solution of the problem. Our interest centred in the matter during the progress of some other work on the water absorption of plants, and on their nitrogenous metabolism, because it suggested the possibility of controlling water content by metabolic methods.

The experimental plants used were radish and turnip (*Brassica Rapa L.*, and *B. Rutabaga D. C.*), but supplementary data are also available for sycamore, dandelion, and *Rudbeckia bicolor*, in which similar conditions obtain. Sand cultures were for the most part used, and the plants were grown in two sets of pots, one set designated Low N, being watered with a solution consisting of Leeds tap-water containing 1–2 mg. of nitrate nitrogen per litre, the second set designated High N, being watered with a solution of tap-water containing 0·1 grm. per litre of nitrate, sodium and calcium nitrates being used in equal proportions. Some cultures were also set up with calcium, ammonium, and sodium nitrates as single salts, and the difference between the high and low nitrogen plants were of the same order as those obtained with mixed salts. Supplementary data were also obtained from water cultures and soil cultures, the results obtained being

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concordant with those derived from the sand cultures. The other environmental conditions were kept constant for both sets of cultures. Expressed sap was obtained by first freezing the plants and then squeezing out the juice in a small press after the manner of Dixon and Atkins. The material for analysis was prepared by drying the tissues well spread out at 95° C. for thirty minutes, and then to constant weight at 65° C. (4). This method proved to be very satisfactory, as none of the leaves became discoloured, and parallel samples gave quite concordant results. Soluble materials were extracted at room temperatures (12°–16° C.) for twenty-four hours in 70 per cent. alcohol, and the residues were then rewashed in 70 per cent. alcohol. Soluble nitrogen and insoluble nitrogen were determined by the Kjeldahl method, amino nitrogen by the micro-method of Van Slyke. Sugars were determined in the cleared solutions after mild hydrolysis and neutralization. This hydrolysis was carried out for ten minutes at 65° C. with 5 c.c. of strong HCl to 75 c.c. of the extract. Hydrolisable polysaccharides were determined in the residue by the same method after hydrolysis by boiling for two and a half hours with 10 per cent. HCl. The estimation of the sugars was carried out by the Schaffer-Bertrand method (9) using a Klett colorimeter. Hydrogen-ion concentrations were determined by the hydrogen electrode and potentiometer checked against standard buffer solutions.

High and Low Nitrogen Metabolism.

In considering the possible origins of nitrogen succulence, the seemingly most obvious thing to suggest is that abundant supplies of nitrogen greatly increase the proportions of protein in the plants receiving them, and since proteins have often been assumed to imbibe large quantities of water in the tissues, to infer that the high water content of these high nitrogen plants is due to a higher protein content. Now it is clear that the gross amount of protein formed in plants receiving abundant nitrogen is higher than in specimens cultured with meagre supplies. The former grow at a faster rate and manufacture much more protoplasmic protein in a given time. Analyses show, however, that high nitrogen plants usually, if not always, contain smaller relative proportions of protein, while low nitrogen plants are proportionally richer in protein. To illustrate these points and the types of results obtained, typical analyses of swede and radish plants are given in Table I as percentages of the dry weight.

Assuming that proteins in the tissues may act as colloids imbibing water, an examination of the above data will make it clear that high nitrogen succulency cannot be due to greater proportions of protein material. There are, however, certain constant differences in nitrogen metabolism. It will be noticed that the ratio of protein to soluble nitrogen is always lower in plants receiving abundant nitrogen, which means that

they have relatively larger amounts of soluble nitrogen. The amino nitrogen is always higher in the high nitrogen plants, and apparently the amide nitrogen is higher as well. Finally, there is a difference in the proportions of chlorophyll in the two types. The high nitrogen plants contain larger proportions of chlorophyll as comparable acetone or alcoholic extracts of the plants show very clearly. Thus, the high nitrogen forms are relatively richer in soluble nitrogen, particularly so in amino nitrogen, amide nitrogen and chlorophyll, and if nitrogen succulence is to be related to nitrogenous material in the plant, it must be due to these or to similar soluble nitrogenous bodies.

TABLE I.

	pH Value Expressed Sap.	Insoluble N.	Soluble N.	Amino N.	Amide N.	Total Sugars.	Hydrolisable Polysaccharides.	Titratable Acidity.	Water Content per cent. Fresh Weight.	Ratio $\frac{\text{Protein N.}}{\text{Sol. N.}}$
Radish, Low N	5·80	3·86	0·67	0·121	0·028	5·18	13·24	28[1]	92·57	5·76
5 weeks. High N.	5·92	2·28	1·38	0·215	0·058	3·72	11·62	33	93·64	1·65
Radish, Low N.	5·31	3·05	0·50	0·172	—	—	—	29	92·71	6·10
2 weeks. High N.	5·59	1·50	0·32	0·257	—	—	—	21	94·47	4·68
Swede, Low N.	5·70	2·71	0·473	0·051	—	5·28	5·70	—	93·21	5·71
8 weeks. High N.	5·81	2·18	0·449	0·071	—	3·10	3·21	—	94·17	4·85
Turnip, Low N.	5·46	1·32	0·44	0·046	0·09	—	—	40	88·80	3·00
5 weeks. High N.	5·68	3·74	1·95	0·067	0·27	—	—	34	90·55	1·92

RELATION OF NITROGEN METABOLISM TO H-ION CONCENTRATION.

The difference impressed on the nitrogen metabolism by the two respective treatments also becomes evident when we examine the respective hydrogen-ion concentrations of the expressed sap of the two types of plant. The sap of high nitrogen plants has a lower hydrogen-ion concentration than that of plants supplied with scanty nitrogen. In Table I the difference varies from 0·11 to 0·28 of pH, but bigger differences than those have been observed, the greatest being between pH 5·27 (low N), and pH 5·75 (high N) for swede seedlings. This marked difference in the hydrogen-ion concentration of the expressed saps probably originates in relative differences in the quantities of certain metabolic materials utilized by the two types of plant during their process of growth. It has already been shown by Molliard (6), particularly with reference to such plants as radish and sorrel, that plants may manufacture considerably greater quantities of organic acids, especially oxalic, when grown in soils poor in nitrogen. Our experiments show that the total acid (or titratable acidity) obtained by titrating the expressed sap until alkaline to phenolphthalein is usually greater in low nitrogen plants, a result entirely in accord with Molliard's

[1] ccs. N. alkali to 100 grm. dry tissue.

observations. This does not, however, appear to be invariably the case. On occasion, we have found the titratable acidity to be greater in high nitrogen plants, indicating a higher total acid content. As this fact is at variance, both with Molliard's results and with the majority of our own, we have attempted to analyse the case further.

From Table II, it is clear that in high nitrogen plants the amino nitrogen is higher.

TABLE II.

	Low N.	High N.	
Radish.			
pH expressed sap .	5·80	5·92	
Titratable acidity .	28	33	c.c. N. alk. to 100 grm. dry tissue.
Amino N. . . .	0·121	0·215	as per cent. dry weight.
Amino-acid (equiv.) .	8·65	15·40	c.c. N. alk. to 100 grm. dry tissue.
Residual acids . .	19·35	17·60	
Differences . .	+1·75	+6·75	
	Res. acid.	Amino-acid.	
Swede.			
pH expressed sap .	5·70	5·81	
Titratable acid . .	0·70	0·745	c.c. N. alk. to 100 c.c. sap.
Amino N. . . .	0·005	0·007	
Amino-acid (equiv.) .	0·358	0·501	alk. equivalent.
Residual acid . .	0·342	0·244	
Differences . .	+0·098	+0·143	amino acid.
	Res. acid.	Amino acid.	

Dissociation (H) of common plant acids (oxalic, citric, malic, &c.) probably × 100 that of amino acids.

Assuming the amino nitrogen to represent entirely or chiefly amino acids, we see that, although the total titratable acidity is greater in the high nitrogen series, the greater part of it is represented by amino acids, which are much more feebly dissociated than the typical plant organic acids. The pH value of the sap will, therefore, be here largely determined by the residual acid, which is much smaller in quantity than in the case of the low nitrogen plants. In the latter case, the total titratable acidity is less, but the amino-acid equivalent much less. It must be remembered, however, that the sap of high nitrogen plants has usually less titratable acidity than that of the other type, and usually higher amino-acid content. In either case the facts are sufficient to account for the higher pH value of the sap. The presence of this abundance of amino acids can also to some extent be explained. An abundant supply of nitrates leads normally to the accumulation of amino acids, which are formed more rapidly than they are used. Their formation leads to a reduction in the gross sugar concentration (see Table I), hence to a reduced organic acid formation, which is the principal cause of the lower hydrogen-ion concentration.

Influence of Hydrogen-ion Concentration on Water Content.

Constantly associated with this difference in the hydrogen-ion concentration of the saps of high and low nitrogen plants, we find a constant difference in the water content of the tissues. Table III gives the results of one series of determinations of these values.

Table III.

		Low N.		High N.		Difference in
		% water.	pH.	pH.	% water.	Water Content.
Radish	4 weeks old	91·90	5·30	5·50	94·61	2·71
„ (Roots)	9 „ „	82·80	5·31	5·75	89·80	7·0
„	5 „ „	92·57	5·80	5·92	93·64	1·07
Turnips	5 „ „	94·41	5·36	5·58	95·72	1·31
„	7 „ „	93·72	5·61	5·83	94·17	0·45
Swedes	3 „ „	94·4	5·30	5·52	95·72	1·32
„	5 „ „	93·70	5·44	5·70	94·10	0·40
„	6 „ „	92·41	5·52	5·71	92·80	0·39
„	7 „ „	90·27	5·70	5·81	91·14	0·87

The existence of this apparently invariable difference in water content correlated with a similar constant difference in the hydrogen-ion concentration of the sap, suggests that the higher water content of the high nitrogen plants might conceivably be due to difference in colloidal (protein) swelling caused by the difference in hydrogen-ion concentration. In this connexion it is of interest to compare our previous data (1927) upon the effects of different external hydrogen-ion concentrations upon the swelling of various parenchymatous plant tissues, e.g. turnips, swedes, and potatoes. These data indicate quite definitely a point of minimum swelling about pH 5·3–5·4, with a sharp rise on the alkaline side of this point. The data already given in this paper for the values of the expressed sap in relation to the water contents of the whole tissues will be found to give similar results. Thus, in the data for turnip, the water content rises 1·31 per cent. when the pH rises from 5·36 to 5·58, and a further rise in pH from 5·61 to 5·83 is associated with a further rise in water content. In all cases in the range of pH from 5·3 to 5·9, the water content is lower when the pH is lower. As the environmental conditions were not definitely constant during the growth of these seedlings, detailed comparison of results on different dates is not strictly justifiable, since the seedlings were at times under moister or drier conditions, which would presumably affect their water content.

One of the most important factors determining the swelling of plant tissues, either in the fresh or dry condition, may well be the influence of the hydrogen-ion concentration on the contained proteins or other colloids. The position of the iso-electric point of the protein on the pH scale deter-

mines the point of minimum swelling, and since most proteins from plant tissues are iso-electric in the region of pH 4·5 (1 and 7), they show minimum volume at this point. Any rise in pH value on the alkaline side of this point should, therefore, lead to a rise in water content of the protein. The increased water content of plant tissues when the *external* pH value is raised between pH 5·3 and pH 6·0 has not so far been convincingly explained, but this may also be due to ampholytes present in the tissues, and iso-electric between pH 5·3 and pH 5·4. In any case, this rise is similar to the effect of increased *internal* pH value in increasing water content. According to MacDougall and Spoehr (5), amino acids, in the concentrations in which they are present in the expressed sap, increase the swelling of carbohydrate colloids like agar, and thus may act as a supplementary factor in increasing the water content of high nitrogen plants.

Our thesis that the higher water content is due to the swelling of protein or similar colloidal material, is supported by the work of Crist (2), whose careful determinations show clearly that nitrate treated plants have a higher percentage of 'bound' water, and greater hydration capacity in the hydratable compounds of their juices. This property of high nitrogen plants of imbibing larger quantities of water is shown, not only by living tissue, but also by the dried materials. If sets of high and low nitrogen plants are first dried to constant weight, and then exposed to ordinary moist air, we find differences in the respective amounts of water absorbed in a given time of the following order:

	Low N.	High N.	
Radish	12·15	15·83	Gain in wt. %.
Turnip	12·44	17·22	

This shows the difference in affinity for water in the colloidal material of the two types.

An explanation of nitrogen succulence previously suggested by Kraus and Kraybill (3), is that NO_3 ions themselves may have a lyotropic effect upon cell colloids. This hypothesis seems to be ruled out by several considerations. Apart from the fact that lyotropic effects are not observed in solutions of low concentration (e. g. concentrations much less than normal), we have not been able to detect any swelling effect of nitrates upon potato or turnip tissue, using the weighing methods of Stiles and Jorgensen (10). Further, growing plants placed in solutions containing high and low nitrogen respectively, do not immediately show differences in water content or structure. In fact, the most characteristic features of high nitrogen plants only appear in those tissues which have developed after the treatment has begun. The larger size of the parenchymatous cells usually associated with high nitrogen, may be taken as an index of the greater suction-pressure or water-absorbing power of the cells during their period of elongation. This

suction-pressure is the difference between the pressure of the protoplasmic membrane on the walls, and the resistance to extension of the wall itself (turgor pressure). We have already given grounds for believing that the swelling pressure of the protoplasm is higher in the case of high nitrogen plants. Our applications of MacDougall and Spoehr's results further suggests that with abundant amino acids the cellulose walls would be more swollen, and consequently more easily extensible. We are inclined to emphasize the probability of this modification of the cell-walls produced by high nitrogen because of the effect on the rate of transpiration ensuing on this treatment. Plants grown under high nitrogen conditions utilize much less water in a given time. Some unpublished results of Miss E. Ryan may be quoted in illustration of this point. Ivy seedlings grown under high and low nitrogen conditions for three months showed the following rates of transpiration as c.c. of water lost per sq. dcm. of leaf-area. There were ten seedlings in each culture.

	Low N.	High N.
1st period—10 days	$86{\cdot}0 \pm 2{\cdot}1$	$59{\cdot}0 \pm 2{\cdot}7$
2nd period—15 days	$109{\cdot}0 \pm 1{\cdot}5$	$81{\cdot}0 \pm 2{\cdot}1$

A similar observation is also made by Crist. Since Miss Ryan was unable to find any constant difference in stomatal number of aperture, it seems as if this result must be attributed to the nature of the evaporating surfaces, i. e. the walls of the cells inside the leaf. That the cell-walls of high nitrogen plants have a greater water holding capacity seems to be a suggestion quite in accordance with the results of our experiments on water absorption by dried tissue.

Summary.

The evidence presented thus shows, that in plants supplied with abundant nitrates, amino acids tend to accumulate, and these influence the metabolism of the plants in such a way that acid production is reduced, with a consequent reduction in the hydrogen-ion concentration of the sap. Both the high amino-acid content and the higher pH value allow of greater swelling of the protoplasmic colloids, and to this feature the higher water content and reduced transpiration may apparently be ascribed.

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Studies in the Gramineae.[1]

VI. 1. Streptochaeta. 2. Anomochloa. 3. Ichnanthus.

BY

AGNES ARBER, M.A., D.Sc.

With seven Figures in the Text.

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1. *STREPTOCHAETA*.

(i) *Introduction.*

STREPTOCHAETA is one of the genera amongst the Gramineae which have aroused most interest from the morphological point of view. Goebel (13), and Schuster (18), following Čelakovský (7 and 8), have treated it as a phylogenetically old type, showing characters primitive for the family. The descriptions, however, on which these conclusions were based, seem to me incomplete, and I have long wished for an opportunity of re-examining the

[1] For references to the previous papers in this series, which represents the work carried out with the aid of a grant from the Dixon Fund of the University of London, see 1–5 in the Literature cited, p. 53.

[Annals of Botany, Vol. XLIII. No. CLXIX. January, 1929.]

two species which constitute the genus. By the kindness of the Director, and of the Keeper of the Herbarium, the Royal Botanic Gardens, Kew, I have been able to study some dried spikelets of *S. Sodiroana*, Hack. Of the other rare species, *S. spicata*, Schrad., I have been fortunate enough to receive spirit material as well as seeds, most kindly collected for me in January, 1927, by Señorita Maria de Carmo de Bandeira of the Jardim Botanico, Rio de Janeiro, Brazil. I wish to express my warmest thanks to Señorita Bandeira, and also to acknowledge the kind help I have received in the matter from Mrs. Agnes Chase of Washington, and from the British Consul-General, Rio de Janeiro. The seeds sent by Señorita Bandeira were successfully germinated at the Cambridge Botanic Garden, though hitherto, as Goebel (13) records, attempts to grow them in Europe have met with failure.

(ii) *Description.*

(*a*) *Streptochaeta spicata*, Schrad.

In vegetative habit *Streptochaeta spicata*, Schrad. (*Lepideilema lancifolium*, Trin.) diverges strikingly from one's general idea of a Grass, especially if that idea is founded on the Grasses of temperate regions. The leaf blades are broad, and are sharply differentiated from the sheathing base, as will be seen in the seedlings sketched (half the natural size) in Figs. 1, B and C, p. 37. The habit of the plant is also rendered unusual by the fact that the leaf-blades place themselves in a spiral sequence (Fig. 1, C), but this appears to be a secondary development, as, in the tranverse section of the terminal bud of a seedling shown in Fig. 1, D, the leaves are arranged, as usual in the Grasses, on two orthostichies—an arrangement which I have also found in a lateral vegetative bud of a mature plant.

The inflorescence consists of a series of spikelets arranged spirally along the axis. An inflorescence removed from its enclosing leaf-sheaths is shown (half natural size) in Fig. 1, A. The six spikelets originate in a $\frac{2}{5}$ spiral. The spiral placing of the spikelets can also be recognized from the two sections drawn in Fig. 2, A_1 and A_2, p. 38; these are taken from a series from below upwards through part of an inflorescence including three flowers. The awns of the spikelets, which are extremely long and slender, are spirally twisted in their apical regions; at the tip of the inflorescence they are liable to wind closely round one another, and they may remain thus adhering together after the detachment of the spikelets from the main axis.

Figs. 2, B_1 and B_2, show two sections through the base of a spikelet which is arising in the axil of a reduced, non-vascular, axillant leaf. A similar axillant leaf is shown in Fig. 2, c_1, but here its identity might not have been recognized, but for the comparison with B_1 and B_2, since it is carried up by the spikelet, and is not free at the level at which the spikelet is detached. I am inclined to think that this axillant leaf is sometimes reduced to the

point of extinction. Figs. 2, C_1–C_5, represent sections from a series from below upwards through a spikelet. In C_2 the glumes which clothe its base are coming into view; there is an undoubted bud-rudiment in the axil of the first, and a structure which is possibly another such bud in the axil of

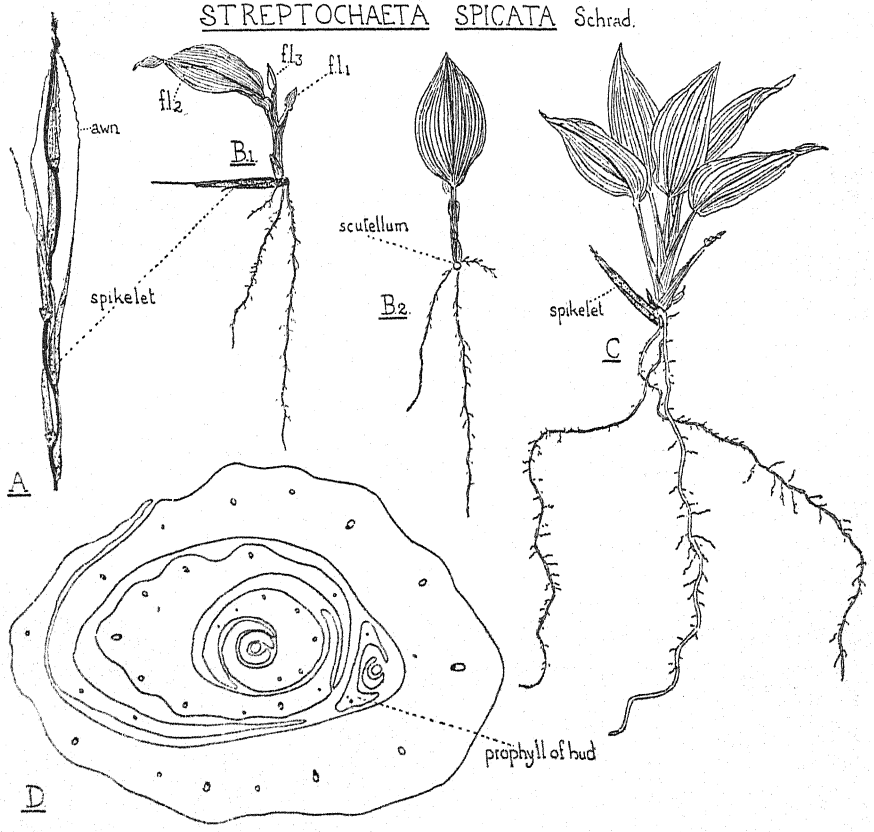


FIG. 1. *Streptochaeta spicata*, Schrad. A, an inflorescence gathered January, 1927, near Rio de Janeiro, removed from its enclosing leaf-sheaths (× ½ circa). It shows six spikelets arranged in a ⅔ spiral; five of the awns are twisted together at the apex. The successive spikelets are dotted, and the main axis indicated in black. B and C, seedlings from seed collected near Rio de Janeiro, January 10, and sown at the Cambridge Botanic Garden, February 21, 1927. B_1 and B_2, drawn on July 6, 1927 (× ½ circa): B_1, *f.l.*$_1$, *f.l.*$_2$, and *f.l.*$_3$, foliage leaves. B_2, view at right angles to B_1, to show small scutellum; the remains of the spikelet, caryopsis, and endosperm have been dissected off. C, plantlet drawn September 27, 1927 (× slightly more than B). D, transverse section (× 45) of an apical bud of a seedling of the same age as B to show that the leaves originate on two orthostichies as in typical Grasses.

the second. Buds in the axils of these glumes were observed by Doell (10). In C_3, all five glumes are visible, that opposite the axis being the last to become detached. This order is not, however, invariable. Fig. 3, A, p. 39, illustrates another spikelet in which the abaxial glume becomes free while two of the laterals are still attached. In these sections, which were cut from young spikelets, I have not found buds in the axils of the later glumes,

but I have observed them in sections of older dried material from Santa Catharina, Brazil.

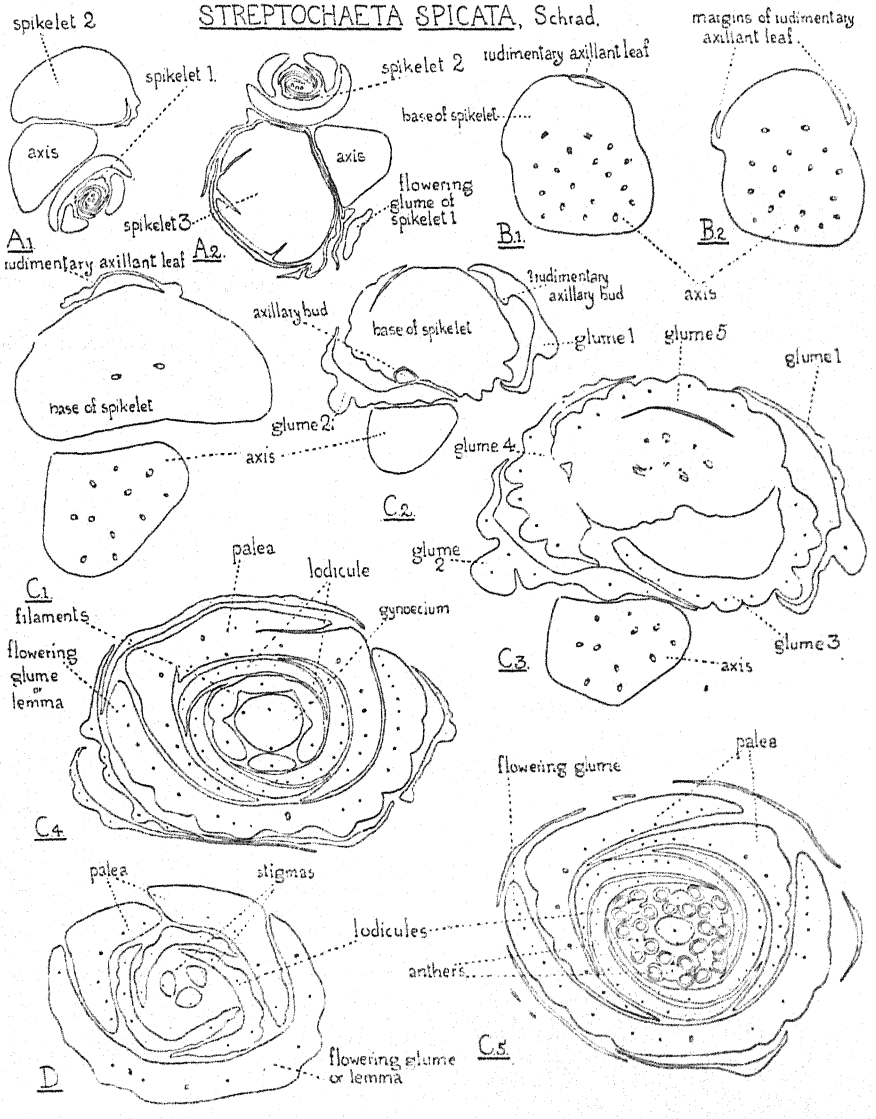


FIG. 2. *Streptochaeta spicata*, Schrad. All figures from material collected January 1927 near Rio de Janeiro. A_1 and A_2, two sections (× 13·7) from a transverse series through part of an inflorescence to show succession of three flowers in spiral arrangement. B_1 and B_2, two sections (× 22) through the inflorescence axis just below detachment of a spikelet to show rudimentary axillant leaf. C_1–C_5, sections from a series through a spikelet, minor axis bundles omitted. C_1, C_3, C_4 (× 22), C_5 (× 22 *circa*), C_2 (× 13·7). D, transverse section through the apex of another spikelet to show stigmas (× 46).

At a slightly higher level the flowering glume, or lemma, and the succeeding organs are differentiated (Fig. 2, C_4); it is the flowering glume

that terminates apically in the long awn which is so striking a feature of the genus. Fig. 3, D_1, shows the region in which the lemma is narrowing into an awn, and in D_2, which is on a larger scale, the awn itself is reached.

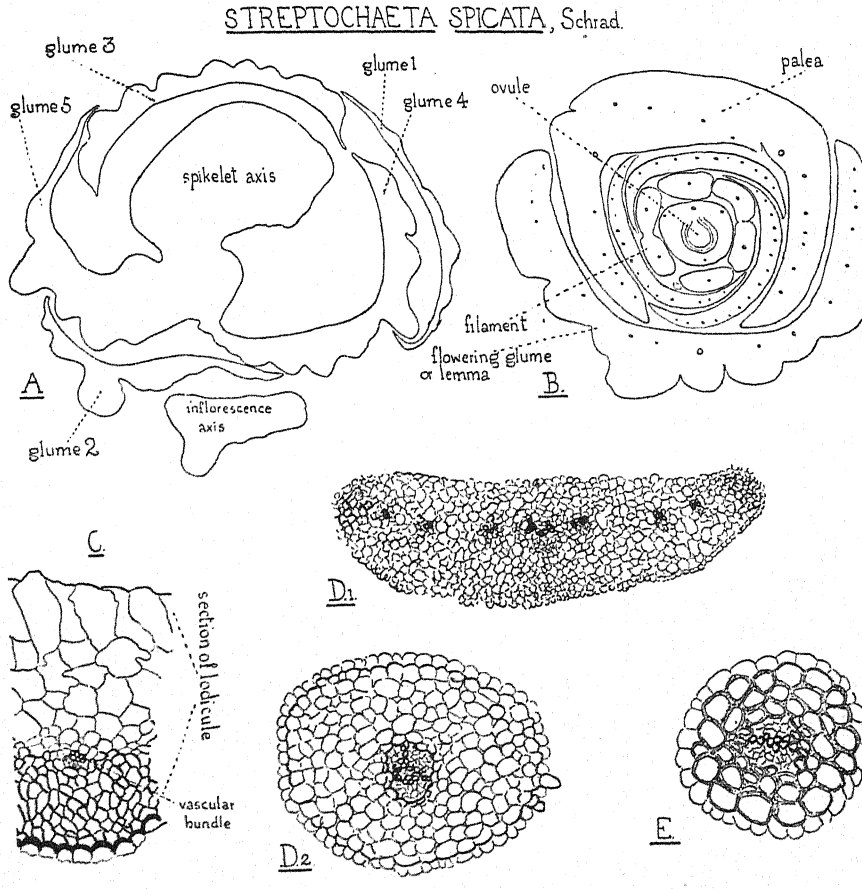


FIG. 3. *Streptochaeta spicata*, Schrad. Except Fig. C, from material collected near Rio de Janeiro, January 1927. A, transverse section (× 22) at the base of a spikelet with glumes numbered according to the order of detachment. B, transverse section of a spikelet (× 22) to show the base of the palea with the two lobes connected. C, small part of a transverse section of a lodicule (× 189), dried material, Santa Catharina, Southern Brazil, (Kew Herbarium); to show large-celled inner tissue and outer zone with thicker walls near the inner side of which the vascular bundles occur. D_1 and D_2, transverse sections through flowering glume or lemma. D_1 (× 75) at the level where it is narrowing to the awn; epidermis partially destroyed. D_2 (× 189) through awn. E, transverse section of another awn (× 311) to show sclerosis of all the tissues except the epidermis and vascular strand.

Hackel (14) describes the awn as 'zarte', but I think that the word is misleading; I find that, at maturity, all the tissue, with the exception of the epidermis and vascular strand, is strongly sclerized, and it appears to be this sclerosis (shown in E, which is more highly magnified than D_2) which is responsible for the spiral contraction of the awn.

Opposite the flowering glume is a leaf which is bifid throughout most

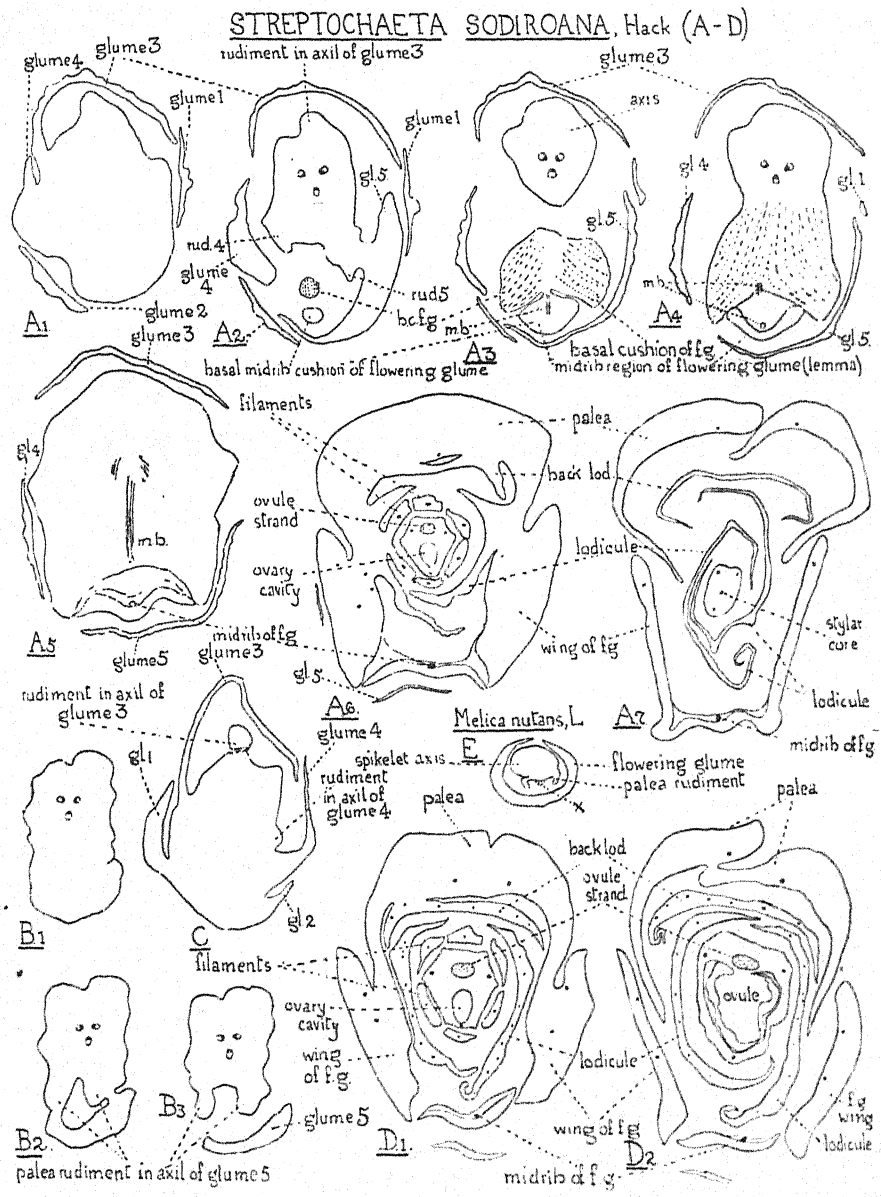


FIG. 4. A–D, *Streptochaeta Sodiroana*, Hack. Figures all from transverse microtome sections (× 23) cut from dried specimens collected by André, 4129, Colombia, S. America (Kew Herbarium). Throughout the figures, *gl.*$_1$—*gl.*$_5$, the sterile glumes preceding the flowering glume or lemma; *rud.*, the rudimentary buds in their axils; *f.g.*, the flowering glume; *b.c f.g.*, the basal cushion of the flowering glume. A$_1$–A$_7$, transverse sections from a series from below upwards through a spikelet. B$_1$–B$_3$, three sections from a transverse series from below upwards through another spikelet below the flower, to show detachment of *glume* 5, and the palea of an undeveloped axillary flower facing it; in B$_1$, the glume is attached, and in B$_3$ it is free. C, transverse section from another spikelet to show unusually well-marked bud-rudiment in the axil of *glume* 3; a minute indication of a rudiment can also be seen in the axil of *glume* 4. D$_1$ and D$_2$, two sections from a series from below upwards through another spikelet to supplement series A$_1$–A$_7$; if D$_1$ and D$_2$ belonged to this latter series, they

of its length (Fig. 2, C_5 and D, p. 38), but at the extreme base its two members are united (Fig. 2, C_4 and Fig. 3, B, p. 38). Provisionally I shall call this organ the 'palea', and assume that it is a single leaf, but its identity will be discussed on pp. 43–5. Inside the palea there are three lodicules, showing a convolute arrangement—a great rarity among Grasses. They are vascular and show more histological differentiation than is usual in these organs; Fig. 3, C, represents a small segment of a lodicule, from a flower older than those from which most of the figures are taken. The lodicules are succeeded by a whorl of six stamens, whose filaments are connected at the base, but for so short a distance as scarcely to justify the term 'monadelphous' which has been applied to them; Doell's description (10), 'filamentis linearibus basi subcoalitis' is more accurate. The gynoecium (Fig. 3, B) contains one ovule supplied by an ovule strand, and possesses in addition an anterior and two lateral bundles—a type of vascular skeleton familiar in the Bamboos (1). The gynoecium narrows to a style (Fig. 2, C_4 and C_5) and terminates in three stigmas (Fig. 2, D).

(*b*) *Streptochaeta Sodiroana*, Hack.

The genus *Streptochaeta* was believed to be monotypic, and all discussion of its peculiarities was based upon the Brazilian species, *S. spicata*, Schrad., until in 1890 Hackel (15) described a second species, *S. Sodiroana* from Ecuador. A few years later his account was supplemented by Čelakovský (8), who dealt with material received from Hackel. Since *S. Sodiroana* is of some interest for comparison with *S. spicata*, I have examined the herbarium specimens available (collected by André in Colombia) as fully as I could. I treated the material by a modification of McLean's method (1, p. 448), allowing six days in dilute potash and thirteen days in paraffin, and I thus obtained serial sections of eight detached spikelets, on which the following account is based.

Figs. 4, A_1–A_7, p. 40, are sections from a series from below upwards through a spikelet. As in *S. spicata*,, five glumes preceded the flowering glume or lemma. The order of detachment of the glumes which I have labelled 3, 4, 5, was the same in all the spikelets examined (six) in which this order could be followed. In A_2, *glumes* 3, 4, 5, are seen each to subtend a minute bud-rudiment; in Fig. C, an example from another spikelet is shown, in which the rudiment in the axil of *glume* 3 is particularly distinct. These rudiments are all non-vascular and extremely small, and they show the tendency to take up gentian-violet which one often notices in degenerating

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would fall between A_6 and A_7. The detachment of the midrib of the flowering glume from its wings is probably artefact. E, *Melica nutans*, L. Transverse section (× 23) of the second abortive flower which succeeded two normal flowers in an inflorescence collected May 16. The flowering glume faces an abortive palea, which never becomes free from the main axis of the inflorescence; the flower itself is represented by a minute patch of degenerating tissue marked × (for comparison with B_3).

tissues; I have observed them in the axils of these three glumes in each of the seven spikelets in which this region was completely cut. Above the five sterile glumes, we come to the lemma—the glume which subtends the only fertile flower. The first sign of its appearance is sufficiently curious. Two small, more or less circular patches of tissue come into view in A_2, within and slightly to one side of *glume 5*. When these are traced up, they are found to represent the basal cushion of the glume. In a former paper (3) I have discussed the basal glume cushions of various Grasses, but that of the lemma of *Streptochaeta Sodiroana* shows an exceptional complexity in that it consists of two parts—an outer cushion which is formed by a downward prolongation of the midrib region, and an inner, which makes a fibrous connecting pad between the spikelet axis and the midrib cushion. In A_3 the two cushions have come into connexion, and in A_4 they are united with the axis. The basal cushion has this double character in seven of my eight spikelets; in the eighth this region cannot be followed owing to the loss of sections. The vascular supply of the glume cushion is best understood by following a few of the diagrams downwards. In A_5, the southern member of the three bundles in the spikelet axis is passing horizontally outwards to supply the midrib (*m. b.*) of the flowering glume; in A_4 it has turned and is running vertically downwards in the outer region of the connecting cushion; in A_3 it is passing into the midrib cushion. From this level it again changes its direction and runs vertically upwards in the midrib; its upward course can be followed in A_4–A_7. In my diagrams the midrib region of the flowering glume appears to be detached from the wing region in Figs. 4 D_1, D_2, &c., but this is probably due only to the loss of the more delicate connecting tissues in the herbarium material used, and the survival of the fibrous tissues alone. I think that this is proved by the fact that I have found a comparable appearance in sections cut from herbarium material of *S. spicata*, although in the spirit material of young flowers of the latter species, from which most of the figures were drawn, the flowering glume was a perfectly continuous structure.

The succeeding organ—the palea—is shown in Figs. 4, A_6, A_7, D_1, and D_2. It is fused basally with the flowering glume (A_6 and D_1), but as soon as it becomes free, its margins are seen to lie within those of this glume. The vascular bundles both in the glume and palea are difficult to detect as they are very small and are hidden by the extreme sclerization of the tissue in which they are embedded. But in the five flowers in which I could best observe them, there appeared to be from one to three small bundles in the median region of the undivided basal part of the palea—two seems to be the most usual number (Figs. A_6 and D_1). A little above the base, the palea bifurcates (D_2 and A_7); in the examples figured one bundle supplies each of the lobes.

Following the palea are three vascular lodicules, which in this species

—unlike *S. spicata*—are so arranged that the back member is external to the two laterals; they are best seen in D_1 and D_2.

I have not observed any special peculiarity in connexion with the six stamens or the gynoecium. The stamens are free to the base as Hackel originally described them (15), and are not monadelphous, as might be supposed from some accounts of the genus. The vascular supply of the gynoecium resembles that of *S. spicata.*

(iii) *Discussion.*

The first morphological point which arises in connexion with *Streptochaeta*, is the question whether the solitary flower is terminal to the spikelet. Goebel (13), when examining the very young inflorescence as a solid object, noticed a prominence opposite to the flowering glume, and identified it as the termination of the spikelet axis. It seems to me that the projection which he saw was more probably the bud in the axil of the uppermost sterile glume. In serial sections of young spikelets I have found no trace of a continuation of the spikelet axis above the flowering glume. I should therefore refer the flower of *Streptochaeta* to the 'terminal' type, of which examples are known among the Grasses, in which the whole apex of the spikelet axis is transformed into the palea and flower, though the flowering glume (or lemma), to which the flower would have been axillant if the axis had continued, retains its place.

The buds in the glume-axils, whose existence I have here recorded, are exceedingly rudimentary, but it seems probable that in the case of the upper glumes they represent abortive flowers. In three spikelets of *S. Sodiroana* the rudiment in the axil of *glume* 5 was found to have a bilobed character (Fig. 4, B_3, p. 40), suggesting the abortive flower of *Melica nutans*, L., which I have figured for comparison (Fig. 4, E, p. 40); the bilobed structure in *Melica* is undoubtedly the palea rudiment which never becomes free from the axis—the flower itself is represented only by the minute patch of degenerating tissue marked ×.

But the chief interest of *Streptochaeta* from the morphological standpoint lies in the interpretation to be put upon the leaves borne upon the spikelet axis below the androecium. These are described by Hackel (14) as consisting of twelve spirally placed members. But even if one accepts the view that the bifid organ which I have labelled 'palea' is really two leaves, inspection of my diagrams will, I think, show that it is impossible to find any system of spiral phyllotaxis with which the arrangement will harmonize. The order of origin of the five first leaves in *S. spicata*, moreover, is not always the same; on comparing Figs. 2, C_3, p. 38, and Figs. 3, A, p. 39, one sees that the abaxial glume which is the last to detach in Fig. 2, C_3, p. 38, becomes free before the lateral glumes in Fig. 3, A, p. 39. In *S. Sodiroana*, my observations on the order of the glumes conflict with those

of Čelakovský (8); I scarcely think that it was practicable to be sure of the succession of these closely placed structures in the days before serial sections were employed for such purposes. I find no identity between the order of the glumes in the two species. In the series belonging to *S. Sodiroana* shown in Fig 4, A, p. 40, the glume which I have called the flowering glume takes its origin only slightly to one side of *glume* 5 which precedes it, whereas in the example of *S. spicata* in Fig. 2, C, it is exactly opposite to *glume* 5. It seems to me, moreover, that the idea of treating the leaves below the androecium in *Streptochaeta* as forming a spiral system, breaks down finally when we come to the lodicules. In *S. spicata* these are convolute from the very base (Fig. 2, C_4, C_5, p. 38, and Fig. 3, B, p. 39), but in *S. Sodiroana*, as Hackel (15) noted in his original description, the scheme is different, and the back member, from the moment of its origin, is entirely external to the two laterals; it is obvious that, of these two divergent arrangements, *both* cannot be fitted into any spiral plan.

I have laid so much stress on what appear to me to be the difficulties encountered if one attempts to interpret the spikelet- and flower-leaves of *Streptochaeta* as forming a spiral series, because this hypothetical spiralness seems to have been the main reason which has led certain botanists to regard the genus as a primitive type. It is true that a tendency to spiral leaf arrangement runs through the vegetative parts of the plant, and is also shown by the arrangement of the spikelets on the axis, but I doubt if this is of any significance; I fancy that broad-leaved Grasses are especially liable to such deviations from the ordinary phyllotaxis of the family.

Another view of the members which succeed the first five glumes must now be considered. Doell (11) maintained that the organs that I have called flowering glume (or lemma) and palea should be regarded as three leaves representing an outer perianth whorl, but, as Eichler (12) points out, this would produce a peculiar sort of perianth whorl (ein sonderbares Perigon), since it includes the large awned glume as one member. No one, I think, in more recent times has supported Doell's view in its entirety, but Čelakovský (8), while taking the awned member to be the flowering glume, has adopted Doell's hypothesis that the bilobed structure which succeeds it represents two perianth leaves. He suggests that these two leaves formed part of an outer perigon whorl, which should theoretically be completed by the presence of a third member in front, opposite the flowering glume (lemma), and Goebel (13) has subsequently reported the discovery of a rudimentary organ in the appropriate position. But as he seems to have observed this rudiment only when looking at the extremely young inflorescence as a solid object, I do not think that much stress can be laid on the observation; no critical results can be obtained on such a point without serial sections.

It must be remembered that the view of Doell, Čelakovský, Goebel,

and Schuster, as to the morphology of *Streptochaeta,* depends entirely on the assumption that the bifid organ succeeding the flowering glume consists in reality of two leaves. It seems to me, however, much more natural to regard the two lobes as the halves of the palea, which is, I believe, in Grasses always a single leaf, though often reduced in the median region and conspicuously bikeeled, as are many other prophylls. I think that the fact that the two halves are united at the base is of some importance in this connexion (Fig. 2, C_4, p. 38, Fig. 3, B, p. 39, Fig. 4, D_2, p. 40); moreover, the vascular system, at least in *S. Sodiroana*, can most easily be interpreted as an example of the familiar palea type with its two main bundles (Fig. 4, D_2); if the two lobes each represented a complete leaf, we should expect their midribs to be more truly median. I recognize that these considerations are in themselves in no way conclusive, but I think that they at least favour the probability of the view which I have adopted in labelling the figures. This view has been recently upheld by Hitchcock (16), who describes the member in question as a palea bifid nearly to the base.

(iv) *Conclusion.*

In conclusion, I should describe the spikelet of *Streptochaeta* as having an abbreviated axis clothed with five crowded sterile glumes, irregularly, and not spirally, placed. These glumes may possibly correspond to two outer empty glumes succeeded by sterile flowering glumes, but this is a point on which one cannot arrive at any certainty. These sterile glumes are followed by an awned flowering glume, or lemma, faced by a palea, which is bifid almost to the base, and which is succeeded by three lodicules, comparable with the three lodicules of the Bambuseae. Doell (10) points out that even the convolute arrangement of the lodicules in *S. spicata* can be paralleled in the Bamboo, *Guadua latifolia*, Kunth. The six stamens and the immature gynoecium show no peculiarity for which we cannot find a term of comparison in the Bambuseae. It will be recognized from this description that there seems to be no basis for Čelakovský's view—which has found support from Goebel and Schuster—that *Streptochaeta* is an extremely ancient type, standing near the original Grass stock. Indeed, it is difficult to see why this genus should have been removed from the position which Doell (10) assigned to it, as constituting a special sub-tribe of the Bambusaceae.

2. *ANOMOCHLOA.*

(i) *Introduction.*

Another rare Brazilian Grass, *Anomochloa marantoidea*, Brongn., has aroused almost as much speculation as *Streptochaeta*; Goebel (13), for instance, has suggested that it is a very ancient type—a survival from a

long past age. *Anomochloa* is a monotypic genus, described by Brongniart (6) in 1851 from material from Bahia[1]. The plant has never been found again. Brongniart points out that at first glance this curious Grass, with its broad-limbed, slender-petioled leaves, with their lax sheaths, exactly recalls

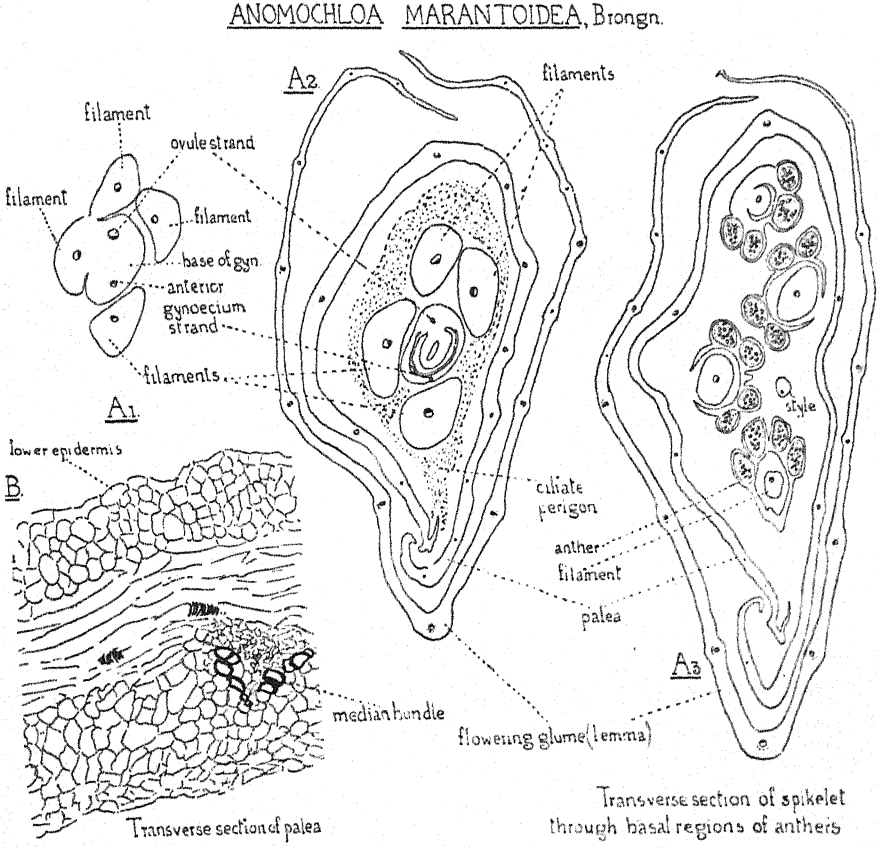


FIG. 5. *Anomochloa marantoidea*, Brongn. The diagrams in Figs. 5 and 6 are all from sections of a herbarium spikelet from a plant cultivated in the Jardin des Plantes, Paris, June 10, 1860. A_1–A_3, transverse sections (× 22 *circa*) from a series from below upwards through the spikelet. A_1, the essential organs only, cut at their extreme base to show the four filaments and the gynoecium. A_2, higher up to show the two glumes and the hairy wreath surrounding the filaments and the gynoecium. A_3, through the style and the basal regions of the anthers, showing various stages of connexion between filament and anther. B, transverse section of the midrib region of the palea (× 189) at the base of the flower below the level of A_1, to show tissue with horizontally running elements, and part of a transverse branch bundle. Epidermis partially destroyed.

one of the Marantaceae—a resemblance which he has emphasized in the specific name. Brongniart's description has been supplemented by Hooker (17), and by Doell (10), both of whom studied specimens grown from seed in the Jardin des Plantes, Paris. By the kindness of the Director, and of the Keeper of the Herbarium, the Royal Botanic Gardens, Kew, I have

[1] Mrs. Chase informs me that this may mean Bahia de Rio de Janeiro, and not, as has been generally assumed, the province of Bahia.

been allowed to examine a single spikelet from the Jardin des Plantes, Paris, dated June 10, 1860. From this spikelet I have been able to obtain over a thousand serial sections, after treating it by McLean's method (1, p. 448). The following description is based on these sections.

(ii) *Description of a spikelet of* Anomochloa marantoidea, *Brongn.*

Two glumes occur immediately below the flower of *Anomochloa marantoidea*. These I have provisionally labelled (in Fig. 5, A_2, and A_3, p. 46) flowering glume (lemma) and palea, but they may equally well represent the outer empty glumes. The 'palea' is not typical in form, as it is not bikeeled. It would not, however, be unique in this respect; the palea, for instance, of the upper flower in the spikelet of the Bamboo, *Oxytenanthera albociliata*, Munro, is not bikeeled (1, p. 457, and Fig. 6, B_2 and B_3, p. 456). In anatomy the 'palea' is somewhat peculiar. The parenchyma near both surfaces is normal in transverse section, but there is an intermediate zone in which the elements are elongated in a tangential direction. This zone, which is shown by Schuster (18, Fig. 23, p. 242), can be seen in Fig. 5, B.

Inside the 'palea' we come to the curious structure, unique among Grasses, which Brongniart (6) described as '*discus* seu *annulus* piloso-fimbriatus'. I cannot add anything to his account of this hairy zone enclosing the stamens, as one cannot safely draw conclusions about its structure from herbarium material. On the assumption that it is equivalent to lodicules, I have labelled it 'ciliate perigon', (Fig. 5, A_2), but it may possibly represent the lemma, in which case the palea and lodicules must be regarded as absent.

The number of the stamens—four—is another peculiarity of the genus (Fig. 5, A_2 and A_3). I am inclined to think that the anterior and the two laterals correspond to the three outer-whorl stamens usually present in the Grasses, and that the extra stamen is the back member of the inner-whorl. In the one flower which I was able to examine, this back stamen was smaller than the others; its filament was 4·35 mm., and its anther (measured to the top of the connective only) was 4·01 mm., whereas the other three had an average filament length of 4·87 mm. and anther length (to the top of the connective) of 4·62 mm., and each of them individually was longer both in filament and anther than the back stamen. I think that these smaller dimensions are consistent with the idea that this stamen is a relic of the inner-whorl, which is present in the Bamboos, but absent in most of the other Grasses. For I have been able to follow the process of reduction of the androecium in some flowers of the Bamboo, *Cephalostachyum virgatum*, Kurz, and I there found that the inner-whorl of stamens was both shorter and more liable to reduction than the outer-whorl, and also that, within the inner-whorl, the back stamen was less liable to be eliminated than the two laterals (2).

Doell (10) mentions that the anthers are nearly basifixed—a rare condition among the Grasses—but I have found no reference to any peculiarity

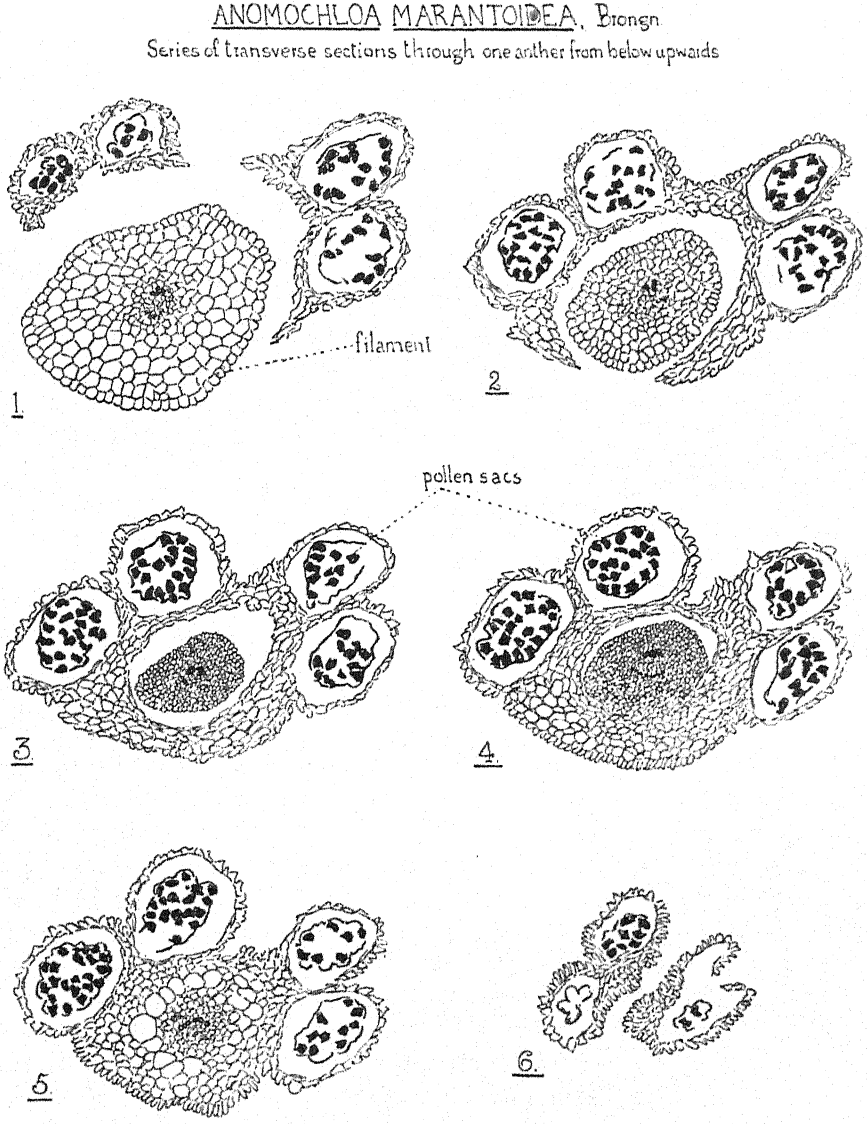


FIG. 6. *Anomochloa marantoidea*, Brongn. Transverse sections from a series from below upwards through the back (northerly) anther in Fig. 5, A (× 77); the anther is not orientated as in Fig. 5, A, but has the dorsal surface downwards.

of form; Schuster (18) who cut serial sections of a spikelet says nothing about the structure of the stamens. But, on examining them in detail, I was surprised to find that, in the form of the base of the anther, and the mode of connexion between anther and filament, they possess a structure for

which I know no parallel among other Grasses. Neither have I been able to match it elsewhere, but I imagine that comparable examples probably exist, though serial sections may be needed to reveal them. The section drawn in Fig. 5, A_3, p. 46, was cut through the lower regions of the four anthers, and it will be noticed that, in the anterior stamen, the anther forms a ring enclosing the filament but free from it, whereas, in the back stamen, fusion has occurred between the ring and the filament; in the lateral stamens, the anther partially encloses the filament. In Fig. 6, p. 48, the history of a single anther can be followed from below upwards. It is that of the back stamen, but it is drawn with its dorsal face downwards, and therefore does not correspond in position with Fig. 5, A_3. (The unnatural appearance of the pollen sacs is due to the contraction of the tapetal layer in the herbarium material used.)

Fig. 6, *1*, shows the base of the anther, with the anther lobes free from the filament and from one another. This phase lasts for 0·18 mm., and the lobes then fuse on the inner side of the filament, and develop marginal wings which gradually approach one another round the back of the filament (Fig. 6, *2*,). The distance for which the lobes are fused but do not meet completely round the filament is 0·66 mm. Fusion of the wings next takes place, and for a short distance (0·24 mm.) the anther forms a free ring round the filament (Fig. 6, *3*). Fusion then begins at the back between the filament and anther (Fig. 6, *4*), and is completed rapidly, in a distance, namely, of 0·10 mm., giving an anther of normal appearance in transverse section, though the connective is more massive than is usual in Grasses (Fig. 6, *5*). This normal stage persists through 2·83 mm. to the top of the connective. The anther lobes stand up freely for a distance of 0·58 mm. above the connective (Fig. 6, *6*).

The base of the gynoecium can be seen in Fig. 5, A_1, in process of detachment from the base of the filaments. It will be noticed that from its very origin it possesses two bundles only; on following these up they are found to be respectively the posterior ovule strand, and an anterior strand in the wall of the gynoecium (Fig. 5, A_2). The anterior strand passes ultimately into the single stigma (Fig. 5, A_3). I have found a similar vascular scheme in the gynoecium of *Lygeum Spartum*, Loefl (5). Curiously enough, Schuster (18), both in *Lygeum* and *Anomochloa*, describes the gynoecium as possessing *three* bundles, and in both cases interprets the single style as due to the fusion of two lateral styles, each with one bundle. But as far as my observations go, he is incorrect in both cases; the single style in *Lygeum* and in *Anomochloa* is (like that of *Nardus*) supplied by a single anterior bundle and not by two lateral bundles.

(iii) *Conclusion.*

My observations, especially those on the structure of the stamens, which appears to be unique among Grasses, emphasize the isolation of *Anomochloa*. There is nothing in the organization of the spikelet to confirm the suggestion that it belongs to a primitive or generalized type. The gynoecium anatomy, and the ciliate zone, which may possibly be reduced from a hairy lemma, recall *Lygeum* (5) but the question whether these resemblances indicate any real affinity must be left undetermined.

3. *ICHNANTHUS.*

In a former paper in this series, I have drawn attention to the basal wings of the flowering glume in the genus *Ichnanthus*, and figured them for *I. pallens*, Munro, and *I. Ruprechtii*, Doell, var. *glabratus*, Doell (3, pp. 476–478, and Fig. 3, p. 477). But it has since been pointed out to me by Agnes Chase, who has published a critical account of this genus in the course of her work on the Paniceae (9), that in the particular species which I studied the wings are relatively poorly developed; owing to Mrs. Chase's kindness in sending me herbarium material, I have now been able to examine other species in which this peculiar character is much more fully displayed. Figs. 7, A_1–A_7, p. 51, are from sections from a series through a spikelet of *I. panicoides*, Beauv. Fig. 7, A_1, shows the two outer empty glumes, and the lemma (flowering glume) succeeding them, which has a rudimentary flower in its axil (A_2). This sterile lemma shows no outgrowths worthy to be called wing-appendages, though in A_1 and A_2 its margins are seen to form slight projections beyond their attachment to the axis. In A_3 the palea of the abortive flower is a conspicuous feature; the object to the south of it is the internode of the rachilla between the sterile and fertile flowers. In A_4 the basal regions of the wing-appendages of the flowering glume of the fertile flower come into view; here they are adnate to the rachilla, from which the normal part of the glume has not yet differentiated. A_5 shows the non-vascular, rudimentary apex of the rachilla, which I have also observed in the only other spikelet which I cut; the fertile flower is thus lateral and not terminal. The flowering glume has become free from the rachilla in its median region in A_5, while in A_6 it is entirely detached, and for the first time its connexion with the wing-appendages becomes clearly apparent. In A_7 the palea and the parts of the flower are differentiated, while the wing appendages are cut in their upper region, in which they are entirely free from the main part of the glume. They consist of a thick-walled epidermis enclosing thin-walled parenchyma, without vascular bundles.

Figs. 7, B and C, show for comparison the wings of the flowering glume of another species, *I. leiocarpus*, (Spr.) Kth. The detailed structure of part of a wing-appendage is shown in Fig. 7, C; there is a conspicuously

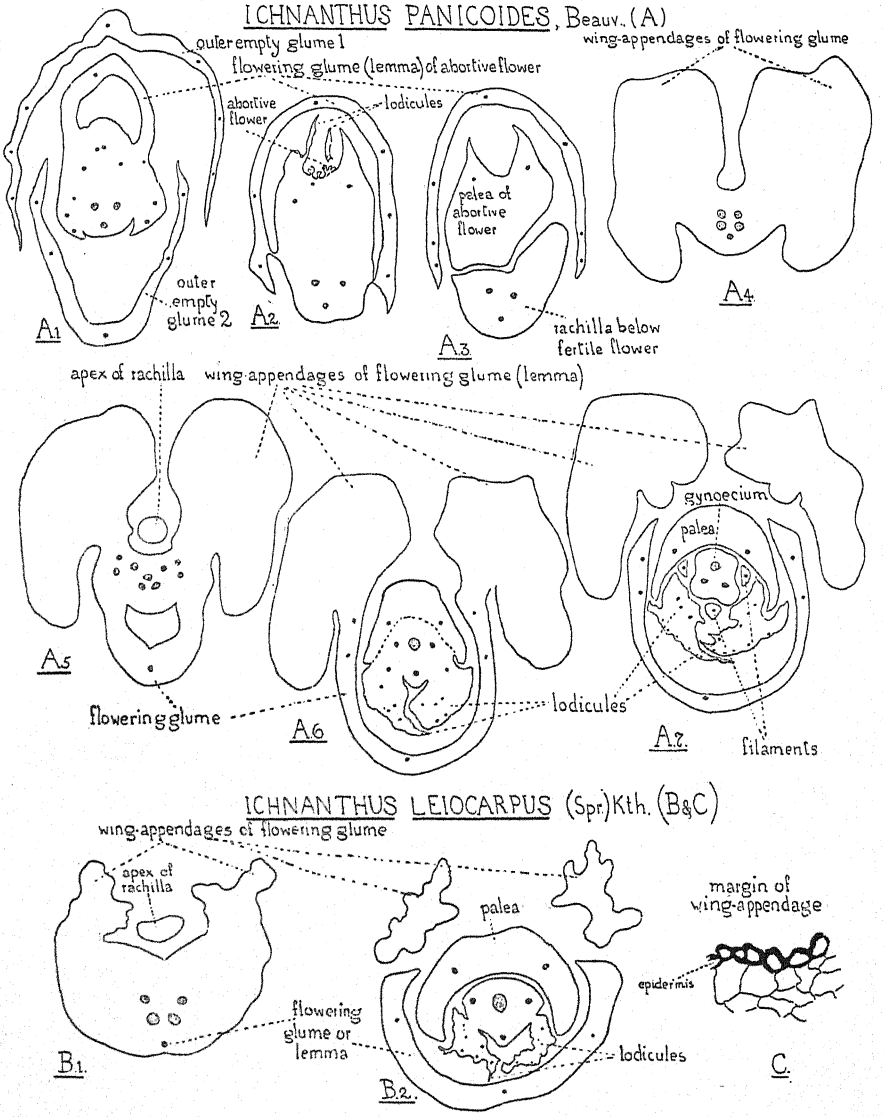


FIG. 7. A_1–A_7, *Ichnanthus panicoides*, Beauv. British Guiana, floor of forest, vicinity of Penal Settlement, west side of Essequibo River, A. S. Hitchcock, 17121, Dec. 6, 1919. Transverse sections from a series (× 22) passing through a spikelet from below upwards, to show the wing-appendages of the flowering glume of the fertile flower, which is preceded by a sterile flower. B and C, *Ichnanthus leiocarpus*, (Spr.) Kth., shady brushy slope, summit of Morro do Urca, Rio de Janeiro, Agnes Chase, Feb. 9, 1925, 8402. B_1 and B_2, two sections from a series from below upwards (× 46) through a flower, to show the appendages of the flowering glume below and above their level of detachment. C, small part of the edge of a transverse section of a wing-appendage at about the level of B_2, to show thick-walled epidermis (× 189). In the case of both species drawn in this figure the herbarium material was not perfectly preserved, especially as regards the lodicules, and the drawings are somewhat reconstructed.

thick-walled epidermis, while the internal elements are larger and thinner-walled than the cells of the main part of the flowering glume. In the one spikelet of *I. leiocarpus* of which I obtained serial sections, the rachilla was continued above the base of the fertile flower as a rudimentary non-vascular papilla (Fig. 7, B_1, p. 51), comparable with that which I found in *I. panicoides*.

4. Summary.

(1) The spikelets of *Streptochaeta spicata*, Schrad., and of *S. Sodiroana*, Hack., are described and figured, and the seedlings of *S. spicata* are also illustrated (pp. 36–43 and Figs. 1–4). From a comparative study of the spikelets it is concluded that *Streptochaeta* diverges less from the other Grasses (especially the Bamboos) than has often been supposed, and that there is no foundation for the view held by Čelakovský and others that it represents a peculiarly primitive and ancient type.

(2) From the study of a single spikelet of *Anomochloa marantoidea*, Brongn., two new points emerge (p. 49). One is that the gynoecium is supplied by two strands only—an ovule strand and a single anterior strand which enters the style (Fig. 5, A, p. 46); such a vascular scheme is known among the Grasses only in *Lygeum* and *Nardus* (5). The second feature of interest is that the stamens possess a structure which seems to be unique among the Gramineae, and for which I have not at present been able to find a parallel elsewhere; in the basal region the anther encircles the filament, so that the former has a ring-like form in section (Fig. 5, A_3, p. 46, and Fig. 6, p. 46). The view suggested by Goebel, that *Anomochloa* is an ancient type, receives no support from the present study.

(3) As a supplement to a former brief account of the wings of the flowering glume (lemma) of two species of *Ichnanthus* (Paniceae) as they appear in serial sections (3, pp. 476–8 and Fig. 3, p. 477), the wing-appendages of two additional members of the genus, in which these structures are developed on a much more conspicuous scale, are described and figured (pp. 50–51 and Fig. 7, p. 51). It is shown that in both species the rachilla continues as a minute non-vascular papilla above the base of the upper, fertile flower, which is thus lateral, and not, as has been sometimes stated, terminal.

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The Biology of Fungus Infection in the Genus Vaccinium.

BY

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With Plate I.

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Introductory.

THE genus *Vaccinium* possesses several features of interest.

The flowers show morphological features that have raised doubts as to the correct systematic position of the genus. In this connexion the isolations by Ternetz (13) of forms of *Phoma radicis*, the mycorrhizal fungus associated with *Calluna*, *Erica*, and *Andromeda*, from species of *Vaccinium*, supports the view that the nearest systematic relationships of the latter are with members of the Ericaceae, while the demonstration of a similar distribution and behaviour of the endophytes in individual species would lend further support to this view.

The fruits of various species of *Vaccinium* are edible and have some commercial value in view of the fact that the plants thrive on acid peaty soils unsuitable for other purposes. For example, *V. corymbosum* has been extensively planted on such soils in the United States (Coville, 1 and 2).

To the writer, the biology and incidence of mycorrhiza is of special interest by reason of the lack of correspondence between Stahl's observations on *Vaccinium* and those on *Calluna* and other ericaceous species. Stahl's conclusions are indicated in the following extract: 'Während manche obligaten Mycorrhizenpflanzen, wie wir früher gesehen haben, der Anzucht aus Samen und der Kultur grosse Schwierigkeiten bereiten, lassen sich die Ericaceen auch ohne Gegenwart von Wurzelpilzen unschwer kultiviren, und ihre Samen gehen, zwar oft langsam, aber in grossem Procentsatz und sicher ohne Mitwirkung symbiotischer Pilze auf.' (Stahl, 12.)

Commenting on the above quotation, and also on the further observation recorded in the same paper that seedlings of *Vaccinium Myrtillus* sown in May on soil which had been previously heated or treated with ether vapour, were found to be free from fungal infection ('völlig pilzfrei') when examined in October of the same year, the following views were expressed by the present writer: 'As a general statement affecting all members of the natural order Ericaceae, the conclusions expressed in the passage quoted above can no longer be accepted. It is true that an obligate relationship with the mycorrhizal fungus cannot at present be predicted with certainty for every member of the group, but, in view of the fact that some species of *Vaccinium* show ovarial infection of the kind described for *Calluna*,[1] a repetition of Stahl's experiments, under conditions in which negative evidence regarding infection of the roots can be more satisfactorily tested than by microscopic examination only, is required' (Rayner 7).

The mycorrhiza of various species of *Vaccinium*, including those used for the present work, was described and figured by Frank in 1887. More recently, Coville (1) contributed an account of that of *V. corymbosum*.

[1] The members of the Vaccinoideae recorded in this paper as showing ovarial infection are *Vaccinium Vitis-Idaea* and *Pentapterygium serpens*.

It is of the usual ericaceous type closely resembling that of *Calluna vulgaris* recently fully described. Individual roots of *Vaccinium* are stouter than those of *Calluna* owing to the presence of a larger number of layers of cortical cells, but the fungal complexes in the outer cells are quite similar in the two genera and call for no further description here (Rayner, 9).

The mycorrhizal fungi of *Vaccinium Oxycoccus* (*Oxycoccus palustris*) and of *V. Vitis-Idaea* were isolated from roots by Ternetz simultaneously with those of *Andromeda polifolia*, *Erica Tetralix*, *E. carnea*, and *Calluna vulgaris* (Ternetz, 12). All the forms isolated, distinguishable from one another by morphological and physiological characters, were included in a new species, *Phoma radicis*, by Lindau and Hennings; those extracted from *Vaccinium*, spp., were named *P. r. Oxycocci* and *P. r. Vaccinii*, respectively. At the time critical proof of their identity with the root endophytes of these ericaceous species was not supplied by Ternetz, but subsequent work has justified the correctness of her attribution in the case of *Calluna* (Rayner, 7) and no reasonable doubt can be entertained in that of the other species mentioned. All were reported to fix atmospheric nitrogen under pure culture conditions in varying amounts, and in the two forms associated with *Vaccinium*, relatively large values were obtained, viz., for *V. Oxycoccus*, 18 mg. of nitrogen, and for *V. Vitis-Idaea*, 22 mg. of nitrogen per grm. of dextrose supplied. The claim for nitrogen fixation by these mycorrhizal forms of *Phoma* has since received indirect support in the case of *P. r. callunae* (Rayner, 6). In view of the unexpected results of the present investigation on *Vaccinium* no useful purpose would have been served by again isolating the endophytes from the roots, and the attempt to do so was accordingly discontinued at a comparatively early stage of the work.

The present research.

With the view of elucidating the exact conditions in respect to fungus infection, experimental work on several species of *Vaccinium* was begun in 1915. Although certain provisional conclusions were forshadowed at an early stage before pressure of war conditions brought the investigation temporarily to a close, its completion has proved unexpectedly laborious, partly because of certain subsidiary difficulties, in particular that of finding an effective method of securing consistent seed germination under laboratory conditions, partly because much of the experimental work yielded inevitably negative results only.

The species used for the greater part of the work were *Vaccinium Oxycoccus*, L., and *V. macrocarpum*, Ait., and the observations now recorded apply indifferently to both these species. *V. Oxycoccus*, the common Cranberry, is a locally abundant plant in peat bogs in parts of Great Britain; *V. macrocarpum* is a North American form closely resembling it, the fruits of which are imported commercially to this country. The two species are

of similar straggling habit and occur in similar habitats, but *V. macrocarpum* has longer and rather coarser branches and larger fruits. A few observations were also made on *V. corymbosum*, the Blueberry, seed of which was kindly sent me by Professor F. Coville. The nature of the work, however, demands ready access to growing material, and since this species does not fruit freely in English gardens, work on it was discontinued. The condition of the ovary and young fruit as regards fungus infection has been investigated in a number of species, fresh material of which became available at various times.

Seed of *V. Oxycoccus* was obtained from various stations in England and Scotland; in the case of both species, seed was available also from cultivated plants and from fruits imported commercially from Russia and North America.

In both *V. Oxycoccus* and *V. macrocarpum*, the fruits are large berries of a fine crimson colour when ripe. They are subject to attack by various parasitic fungi that cause characteristic changes in appearance and texture and bring about reddening or discoloration of the flesh (10). In the sound berry the flesh is firm and white and the colour limited to the outer skin; for this reason it is easy to select fruits free from casual contaminations by parasites, and such clean berries stand the winter in the south of England and may be found with the flesh still firm and white in the early spring. Each fruit is four-chambered with a number of seeds springing from the basal part of an axile placenta. The seeds vary in size from 2·5 mm. to 3 mm. long by 1 mm. to 1·5 mm. broad, each with a brown, slightly sculptured seed coat and a ridge along one side. In longitudinal section each shows the curious chamber at either end described by Peltrisot (4). The seeds are similar in type to those of *Erica* and *Calluna* but of much larger size (Pl. I, figs. 1, 2).

An initial difficulty was experienced in securing satisfactory germination especially during the autumn and winter. Seeds sown in peaty soil in a cool greenhouse germinate irregularly in the autumn, the germination capacity improving towards the spring. Similar seeds sown in germinators under experimental conditions, e.g. on filter paper moistened with rain-water, or on agar media (pH. 3·5–3·8) do not germinate, or give a negligible percentage of seedlings when sown during the autumn and winter; moreover, sown after the middle of February, germination is slow and very irregular, especially in *V. Oxycoccus*. This proved to be the case whether seeds were sown immediately after removal from the fruits, or allowed to become air-dry before sowing. For example, in one germination test of *V. macrocarpum*, 500 seeds, removed from the berries on December 20 and air-dried, were sown in a germinator on May 30 following. As a result, three seedlings were obtained in July, the normal period required for germination being 18–21 days.

In both species the germination capacity is affected adversely by age, e.g. seeds germinate best in the spring and early summer following collection, and show greatly reduced germination capacity a year later. The inconsistent and unsatisfactory germination obtained under laboratory conditions was not affected materially by:

(*a*) Storing fruits in *Sphagnum* over winter in the open.

(*b*) Exposing fruits and seeds to a temperature of 3° C. to 4° C. for varying periods up to eight weeks before placing to germinate.

(*c*) Removing seed coats with or without subsequent exposure to low temperatures.

As it was desirable for experimental purposes to be able to germinate seeds on artificial media at any season, and essential to do so in order to eliminate the possible lethal effects of various sterilizing agents, considerable time was devoted to discovering a satisfactory technique. It was found eventually that preliminary exposure of seeds to ether vapour facilitates germination at any season without adverse effect on the resulting seedlings.

The procedure found most satisfactory is as follows:

Seeds are exposed to an atmosphere saturated with ether in a desicator for twenty minutes, placed in a current of fresh air for half an hour or so, and immediately afterwards placed under favourable germination conditions. The latter is important and it was noted that seeds kept dry for any considerable time after treatment were affected adversely. Etherizing also improves the germination capacity of old seeds. The following are records of actual observations from a seed test carried out in the middle of June on filter-paper moistened with rain-water:

Species.	Description of Seed.	Percentage Germination.
V. Oxycoccus	2nd season, untreated	No germination.
,,	,, ,, etherized 20″	1 %
,,	1st ,, untreated	10 %
,,	,, ,, etherized 20″	40 %
V. macrocarpum	2nd ,, untreated	5 %
,,	,, ,, etherized 20″	20 %
,,	1st ,, untreated	98–100 %
,,	,, ,, etherized 20″	98–100 %

Similar difficulty in germinating seed of various species of *Vaccinium* has been experienced by other workers both in this country and in Russia.

The cause of dormancy cannot be stated with certainty, but it is clear that the capacity to germinate is affected by the character of the substratum. These species of *Vaccinium* grow naturally in acid peat and it is possible that the improved germination capacity noted when seeds are sown in untreated peat may be connected with changes in the seed coats and (or) endosperm induced by contact with acid humus. Observations on the

behaviour of seeds placed to germinate on acid agar media make it improbable that the hydrogen-ion-concentration of the medium is the only factor involved, but, in view of Knudson's observations on the response shown by Orchid seeds to small variations the hydrogen-ion-concentration of the substratum, this matter requires more detailed study (Knudson, 3).

The problem inviting solution in *Vaccinium* may be analysed as follows:

(*a*) In what degree, if at all, is seed of *Vaccinium* infested with mycelium of the mycorrhizal fungus associated with the species concerned?

(*b*) Is it possible by the use of sterilized seeds to raise and cultivate seedlings free from infection?

(*c*) Does an obligate relation, analogous to that found in *Calluna*, exist in *Vaccinium*? If so, at what stage in the development of the seedling is it established?

In view of recent observations on *Calluna* there may be added:

(*d*) Is it necessary to distinguish between *fungus infection* and *mycorrhiza formation* in *Vaccinium* as in *Calluna*? If so, what rooting conditions are favourable or otherwise to the development of normal mycorrhiza in the root system of the former?

(*a*) *Seed Infection.*

Examination of very young cranberry fruits in July by means of longitudinal sections yields indubitable evidence of ovarial infection. Mycelium is present, though scantily, in the ovary chambers and associated with the developing seeds; the hyphae are hyaline and of extreme fineness Hyphae of similar character are present in the inner tissues of ripe fruits but are difficult to demonstrate externally upon seeds after removal from the berry. Owing to their liability to attack by a number of parasitic fungi,[1] the greatest care must be exercised in selecting berries for examination. The photograph reproduced relates to sound fruit entirely free from extraneous infection of any kind (Pl. I, Fig. 5).

Examination of sections through seeds placed the fact of seed-coat infection beyond doubt. Fine colourless hyphae are present, not only upon the outside of the testa, but throughout the several layers of tissue composing it, extending to the most deeply situated cells in contact with the endosperm (Pl. I, Figs. 3, 4, 6).

Very puzzling experimental results were obtained when attempts were made to obtain a pure culture of the endophyte from the mycelium present in the fruit tissue and seed coats. For the sake of brevity a full account of the experimental details is omitted and the general results summarized as follows:

[1] Several of the parasites mentioned by Shear as occurring commonly on fruits of *V. corymbosum* in the United States, e. g. *Guignardia vaccinii* have been noticed upon berries of *V. Oxycoccus* and *V. macrocarpum* from time to time.

Seeds removed aseptically from sound, superficially sterilized fruits behaved as if sterile when plated upon various media, e.g. nutrient agar containing dextrose and peptone, *Vaccinium* fruit-extract agar, *Rhododendron* root-extract agar, *although subsequent examination proved that mycelium was present in the tissues of the seed coats.*

In the case of seeds treated in this manner in the autumn and removed from the seed dishes for microscopic examination after several months, a certain degree of activity of the mycelium present in the cells of the testa has been noted. In no case has mycelium been observed to grow out into the surrounding medium.

As a result of repeated observations of a similar kind extending over a number of years, it was concluded that there is regular and deep-seated infection of the seed coat and a general coincidence between dormancy of the seed and a corresponding inactivity of the mycelium associated with it. The growth of the embryo at germination is accompanied by a corresponding renewal of activity of the part of the mycelium resulting in a regular infection of the seedling tissues at germination (see Pl. I, Fig. 2).

(*b*) *Sterilization of Seed.*

Various difficulties presented themselves in devising a satisfactory technique for seed sterilization.

Owing to the character of the outer wall of the testa, seeds are not easily wetted by liquid disinfectants, the difficulties of manipulation being increased by the presence of two relatively large air chambers in the seed coat (Pl. I, Fig. 1). In the case of *Calluna*, difficulties of a like kind were overcome partially by centrifuging the seeds before sterilization, a procedure ineffective in *Vaccinium*, owing to the larger chambers and increased buoyancy.

After preliminary trial with a number of sterilizing agents, 1 per cent. mercuric chloride was selected as the most effective. The seeds were treated with ether vapour, and then immersed in the sterilizing agent for 5 to 10 minutes, various measures being adopted to secure thorough wetting of the testas. After treatment they were rinsed repeatedly with sterilized water and sown upon nutrient agar. In course of time, a number of cultures was obtained free from external contamination, the seedlings showing normal germination capacity as measured by that of controls in peaty soil. Seedlings raised in this way were subsequently transferred from the seed dishes to culture tubes containing respectively a complete solution of mineral salts in agar, peat-extract agar, and *Sphagnum*-extract agar. All these seedlings developed healthy shoots with six to seven leaves; some remained practically rootless, others produced short roots in the agar medium, the majority gave rise to transparent adventitious roots from the upper parts of the stems (Pl. I, Figs. 7, 10). The roots of such seedlings invariably yielded evidence of fungal infection when examined microscopically.

After repeated trials, this method of experimentation was found to give inconsistent and unsatisfactory results with regard to infection of the seedlings. In view of the observed fact that the mycelium extends to the innermost layers of the relatively massive seed coat, the attempt to raise seedlings free from infection by the classical method of seed sterilization was abandoned and a new technique devised.

The comparatively large size of the seed in these species of *Vaccinium* and the possession of a relatively thick testa renders it possible to remove the seed coat without injury to the endosperm and contained embryo. With practice, the operation can be performed quickly and, if carried out skilfully under aseptic conditions, it is practicable to sow seeds so treated upon nutrient agar without contamination. The culture vessels used were small flasks of 100 c.c. capacity, in each of which were placed 25 c.c. of agar nutrient containing salts, sugar, and peptone.

The exact procedure was as follows: after preliminary treatment with ether vapour, the seeds were rapidly sterilized, rinsed in sterile water and transferred to a flamed slide. The seed coat was then quickly removed by means of sterile instruments and the contents transferred with a flamed platinum loop to the culture flasks, six seeds being placed in each vessel. The whole process was carried out in a small, carefully sterilized chamber, due aseptic precautions being observed by the manipulator. In all, 150 seeds were successfully treated in this way and transferred to twenty-five culture flasks. In no single case did casual contamination occur in these cultures, and the contained seeds gave a germination capacity of 95 per cent. Of the seedlings so produced, a small proportion did not develop chlorophyll, owing apparently to premature loss of contact with the endospermic mass during germination due to mechanical causes following upon the removal of the more or less rigid seed coat. The majority produced normal healthy seedlings that developed several leaves while still in the seed dishes. Transferred to culture tubes of various media, e. g. solutions of appropriate inorganic salts in agar, extract of peat in agar, extract of *Sphagnum* in agar, sterilized peat, &c., many of them grew into vigorous plantlets with a well-developed shoot system. In general, they did not root freely in any of the media supplied and showed marked browning of the young rootlets. On the other hand, they commonly formed perfectly healthy transparent roots above the surface of the medium (Pl. I, Figs. 8, 9, see also p. 61).

Whether this refusal to root freely depends solely upon the reaction and constituents of the substratum has not been fully investigated.

All such ' pure culture ' seedlings, at whatever stage examined, showed sparse infection of roots and shoots by fine mycelium. One seedling of *V. Oxycoccus*, transferred to sterilized peat and watered with sterilized rain-water, was grown for two years and three months from the time of sowing.

It formed a normal, well-rooted plant, the clean, transparent roots of which showed scanty infection by fine hyphae without formation of mycorrhiza (Pl. I, Fig. 17). The type of infection observed is identical in every way with that exhibited by *Calluna* when 'pure culture' seedlings containing the mycelium of the endophyte are grown in sterilized substrata of various kinds. It is evident that the formation of functional mycorrhiza in *Vaccinium*, as in *Calluna*, is conditioned by the nature of the rooting medium.

These experiments provided a sound basis for the following conclusions:

(*a*) Seeds of *V. Oxycoccus* and *V. macrocarpum* are contaminated by mycelium while still in the fruit, such infection affecting not only the seed coat, but also the tissues of the seed itself. That the mycelium present belongs to the mycorrhizal fungus proper to the species may be inferred from its mode of occurrence, structural features, and relations with the host tissues, as also by its rapid extension to the roots in young seedlings germinated under aseptic conditions.

(*b*) It is, therefore, impossible to raise seedlings free from fungal infection by the use of superficially sterilized seeds. However drastic the method employed, they will always be subject to infection from mycelium present in the endosperm, if, indeed, the cells of the embryo have not already suffered invasion in the resting seed.[1]

Consequently,

(*c*) It is not practicable to determine experimentally whether or not there exists in *Vaccinium* an obligate relation of the endophyte with seedling development analogous to that known in *Calluna*. The classical method of demonstrating this relation by comparing the behaviour of 'pure culture' seedlings with that of similar seedlings brought into contact with the appropriate fungus is here not available.

The experimental evidence thus obtained provided welcome confirmation of suspicions long entertained respecting the existence of extensive seed infection, and offered a new and encouraging starting-point for re-examination of the seed tissues in pursuit of satisfactory ocular evidence—hitherto lacking—of their extensive invasion by mycelium.

In ericaceous species, demonstration of the presence of mycelium throughout tissues other than those of the root is a matter of considerable difficulty. The mycelium is extremely reduced and difficult to stain. It is closely associated with the cell-walls, passes freely in and out of the cells, and, when present in relatively large strands in cells or intercellular spaces,

[1] The possibility of sterilizing seeds effectively by preliminary treatment with hot water at a temperature lethal to the mycelium but not to the embryo has not been tested. This method has been used successfully for seeds of *Pavetta*, sp., infected by the strains of *Pseudomonas radicicola* associated with leaves of the mature plant. The less obvious character of seedling infection and the inevitably negative nature of the evidence for non-infection render this and similar methods unsuitable in the case of *Vaccinium*.

is liable to be washed away by manipulation of the sections. Its presence and distribution in *Vaccinium* have been established beyond doubt only by the elaboration of a special technique and the provision of optimum optical conditions. It is believed that mycelium is closely associated with the cell-walls, but it has been found impossible to demonstrate this clearly by means of differential staining. Where it occurs free, mycelium stains with many of ordinary stains, but it is extremely liable to become detached and float away during manipulation of the sections or slide. On the whole, the most satisfactory results have been obtained by the use of fairly thick sections mounted directly in lactic-phenol; several of the arlier photomicrographs providing critical evidence of fungus infections were obtained from sections prepared in this way. More rarely, convincing evidence can be obtained by the use of microtome sections stained in various ways. Among the stains used were Heidenhain's haematoxylin, Orseillin-aniline blue, Haematoxylin-eosin, Acid-fuchsin, Malachite green with Martius gelb, and various modifications of the lactic-phenol-aniline blue method. For the reasons stated, none of them is invariably successful, and any of them may occasionally give excellent results. Immersion of the sections in lactic-phenol is almost invariably of service, but the removal of contents by solution decreases the stainability of the mycelium with certain stains. In general, the advantages of staining are overbalanced by the risk of loss of fragments of mycelium during manipulation. The photomicrographs reproduced (Pl. I, Figs. 3, 4, 5, 6, 11, 12, 13, 15) provide final and conclusive evidence of the presence of fine mycelium in tissues of the fruit, seed coat, endosperm, and seedling shoot in *V. Oxycoccus* and *V. macrocarpum*.

Ovarial infection has been observed also in the flowers of *V. Vitis-Idaea* (Rayner, 5) *V. Myrtillus*, *V. pennsylvanicum*, *V. ovatum*, *V. vacillans*, and *V. corymbosum*, connoting a similar distribution of hyphae throughout the plant in each case. The exact period at which the hyphae appear within the ovary chambers has not been fully investigated, and does not appear to have any special significance. Here, as elsewhere, mycelium is difficult to put in evidence, but here, as elsewhere, a prolonged search invariably has yielded positive results. . . . In *V. macrocarpum* mycelium is present in the young fruit-chambers in July, and has extended to the endosperm of the seeds by the middle of August at a stage of development just coincident with the completion of wall formation.

Fungus infection of the seed.

The ripe seed has a relatively thin testa consisting of five to six layers of cells. The external and radial walls of the outermost layer are colourless and have undergone change to a substance giving pectose reactions; the remaining walls are cuticularized and pitted, the cell cavities of the innermost layers compressed and flattened by tangential stretching. Each

seed has a thickened ridge along one side, and at either end the curious cavities described by Peltrisot (4). In the ripe seed, these chambers contain massive cellulose bars and granular protoplasmic remains derived from the earlier stages of development. Mycelium is not usually plentiful upon and within the seed coat—the condition shown in Fig. 3 is exceptional in this respect—but it is not difficult to obtain evidence of the presence of fine mycelium, sparsely distributed throughout the cells. The hyphae are fine, hyaline, and pass freely through the cell-walls without any sign of swelling or constriction. They may be found at any depth from the surface extending to the flattened cells of the inner layer (Pl. I, Figs. 3, 4, 6).

The cells of the endosperm contain abundant reserves in the form of oil and large protein granules. Imbedded in this relatively massive tissue, and extending about two-thirds of its length, is the small embryo —an undifferentiated axis with two small lobed cotyledons. During early observations on *Vaccinium*, strands of fine mycelium were observed more than once in tantalizing proximity to the endosperm tissue, but conclusive evidence respecting the origin and distribution was not forthcoming. Owing to the nature and abundance of the reserves, sections of the fresh seeds are extremely opaque, and the necessary clearing processes are usually effective in removing the more obvious signs of mycelial infection. Convincing evidence of its existence is most readily obtained in sections of fresh seeds cut in gum with a freezing microtome and mounted immediately in lactic-phenol. From the nature of the case, hyphae are more difficult to demonstrate in microtome sections, but in either case fortunate preparations leave no doubt of the penetration of the endosperm tissue by hyphae, or of their extension to the immediate neighbourhood of the embryo. They have never been observed within the tissues of the latter (Pl. I, Figs. 11, 12, 13). Confirmatory evidence of the presence of mycelium in the endosperm was secured by sectioning early stages of germination in seedling cultures obtained by aseptic removal of the seed coats. A vertical section through the germinating embryo and partly depleted endosperm of one such seedling at a stage just subsequent to emergence of the radicle is shown in Pl. I, Fig. 14; thinner sections yielded ample evidence of fungus infection (Pl. I, Figs. 12, 13).

Although mycelium has not yet been observed to grow out into the medium in these aseptic cultures of *Vaccinium*, and it is believed that the embryo is immune to infection in the resting seed, hyphae quickly penetrate the tissue of the emerging seedling at germination. Pl. I, Fig. 16 illustrates a longitudinal section through the hypocotyl of one of the 'pure culture' seedlings illustrated in Pl. I, Fig. 9, showing locally abundant fine mycelium in and about parenchyma cells of the cortex. In rather cruder fashion, the same phenomenon can be observed in seeds of *V. Oxycoccus* and *V. macrocarpum* removed from sound fruits and germinated under

aseptic conditions; it is illustrated in *V. corymbosum*, the Blueberry, in (Pl. I, Fig. 1).

At present it cannot be definitely stated whether the observed inability of the mycelium to grow out from seeds or other tissues of *Vaccinium* into the surrounding medium is determined solely by the constitution and reaction of the substratum. In Ling (*Callus vulgaris*) isolation of the endophyte from seeds removed aseptically from unopened fruits has been accomplished repeatedly, but always with difficulty, i. e. only a very small proportion of the seeds plated yield colonies of the fungus, although in the case of those apparently sterile, the presence of mycelium in the fruit cavity has been confirmed repeatedly by microscopic examination of seeds from the same fruits. In *Vaccinium*, mycelium has never been observed to grow out into the surrounding medium, although in view of the known distribution throughout the tissues, special efforts were made to obtain cultures by plating slices of the fruits and seeds (aseptically removed from ripe fruits) on many different kinds of media. There is certainly a very close relation between the behaviour of untreated seeds in respect of germination and the constitution of the seed-bed, as there is also apparently between the formation of roots by 'pure culture' seedlings and the constitution of the rooting medium. These matters and their relation, if any, with behaviour of the endophytic mycelium in respect of independent growth outside the tissues require fuller investigation.

The wide distribution of mycelium throughout the tissues in *Vaccinium* has been confirmed experimentally by rooting shoot cuttings under controlled conditions. A plant produced in this way and subsequently cultivated for seven months in sterilized peat watered with sterilized rain-water showed sparse infection by fine hyphae of exactly the same kind as that described in seedlings raised and grown under aseptic conditions (see p. 62).

(*d*) Finally, in *Vaccinium* as in *Calluna*, it is necessary to insist on the distinction between fungus infection and mycorrhiza formation. The former takes place regularly at the earliest stage of development; the latter is an annual event, influenced largely by soil conditions. From the nature of the case it is unfortunately impossible to compare directly the growth of individual plants of *Vaccinium* containing mycelium of the endophyte with that of others free from such infection, and therefore likewise impossible to prove definitely that the association is invariably beneficial to the vascular hosts. The old definition, *obligate mycorrhiza plants* as compared with *facultative mycorrhiza plants* can be directly applied to these ericaceous species in the sense that the appropriate endophytes are invariably present in their roots. It must not be assumed to imply that individual plants depend upon the formation of typical mycorrhiza to ensure healthy vegetative growth.

Discussion of Results.

The research in *Vaccinium* passed through three phases. Preliminary work pointed to seed infection of a more deep-seated character than had hitherto been observed in Ericaceae and provided a satisfactory method of ensuring satisfactory germination under experimental conditions at any season. The attempt to raise 'pure culture' seedlings either by methods of seed sterilization or by aseptic sowings of seeds deprived of their seed coats proved fruitless. It supplied, however, the necessary experimental proof of suspected fungus infection within the seed tissues and led finally to the provision of satisfactory ocular proof of such infection.

Species of *Vaccinium* are among the more specialized members of the Ericaceae in respect to soil conditions, and it is perhaps not surprising to find represented among them the most extensive and intimate association between vascular host and endophytic fungus yet discovered in the family. Were the development of mycelium less repressed in the vegetative tissues, it would be easy to succumb to the temptation to describe such species as *Calluna vulgaris* and *Vaccinium*, spp., as lichenoid in structure.

As at present known, the case of *Vaccinium* represents the most highly evolved symbiotic relation yet discovered in Ericaceae, and constitutes a unique case of very intimate association in mycorrhizal relationships. Outside the group, the only comparable condition is that recorded in *Lolium* (McLennan, 5) but here several links in the chain of evidence connecting the mycelium in the seed with that in the mycorrhiza are still missing, and there is no allied species to serve as a standard of comparison. In *Vaccinium* it is impossible to prove that fungus infection is indispensable to seedling development, although, by analogy, that conclusion becomes almost irresistable.

The direct relation of mycorrhiza formation to soil constitution is emphasized once more, and in *Vaccinium* there can be little doubt of its immediate connexion with an abundant supply of natural humus. Whether the observed inhibition of mycorrhiza formation in sterilized peat is due directly to the production of toxic substances, or to alteration or removal of those favouring its formation in natural peat is not at present known.

It should be possible to supply an aseptic rooting medium which will provide optimum condition for the formation of typical mycorrhiza *Vaccinium* and other ericaceous species under experimental control. Artificial tree mycorrhizas resembling those formed in nature have been obtained by Melin, who has also offered experimental evidence of their functional activity. It is significant, however, that these were not formed in large numbers in Melin's cultures, and this observer has drawn attention to the toxicity of heat-sterilized humus to the mycorrhizal fungi of trees (Melin, 6).

From the evidence provided by my cultures it is apparent that in *Vaccinium* and its allies, mycorrhiza formation is likewise hindered by certain conditions in the rooting medium, and it may be inferred that in nature it is promoted by an abundant supply of suitable organic material in the soil, a view in complete accord with that reached by observation in the field.

The evidence derived from experimental work on ericaceous species, leads inevitably in my opinion to the conclusion that functional mycorrhiza is beneficial when the host plants are growing in the humus soils in which it is typically developed, independently of possible benefit due to the endophytes as facultative nitrogen fixers.

It has also established with certainty the crucial fact that under the relatively sheltered conditions of experiment the development of mycorrhiza is not essential for free and vigorous vegetative growth, even taking into account the remarkable fact that practically all organs of the plant are permeated by mycelium of the endophyte.

The bearing of this latter fact on the question of nitrogen fixation by these ericaceous endophytes is obvious and emphasizes the importance of obtaining final proof with regard to this matter.

With regard to the conclusions of Stahl (12) cited on page 56, it is clear that the experimental evidence justifies the earlier views expressed by the present writer (p. 57). In view of the deep-seated infection of the seed of *Vaccinium* and the evidence yielded by experimental cultures, both of sterilized seeds and of seeds subjected to aseptic removal of the seed coat before germination, it is impossible to accept Stahl's statement that plants grown in treated soil were 'völlig pilzfrei' after five months' growth. The fact of root infection was overlooked by Stahl—as more recently by Christoph in the case of *Calluna*—because the formation of mycorrhiza is partially inhibited in the roots of both species when grown in a sterilized medium. Under these conditions, the demonstration of mycelium in the mycorrhiza cells of the root requires a more careful technique than was bestowed upon it by either of these observers.

Summary.

1. Mycelium of the endophytic fungi of *Vaccinium Oxycoccus*, L., and *V. macrocarpum*, Ait., is present throughout the shoot tissues extending to the ovaries and other organs of the flower.

2. Seeds of these species of *Vaccinium* are subject to infection by their respective endophytes while still within the fruit chambers.

3. Infestation of the seed is more extensive in *Vaccinium* than in *Calluna*. Mycelium is present throughout the tissue of the seed coat and penetrates deeply within the endosperm of the resting seed. At this stage it has not been observed within the tissue of the embryo.

4. Owing to the character of seed infection it is not possible to obtain seedlings of *Vaccinium* free from fungus infection, whether by means of seed sterilization, or by removal of the seed coats previous to germination. At germination, all tissues of the emerging seedling are subject to invasion by fungus mycelium. Although not susceptible of direct proof, it may be inferred that normal seedling development is bound up with such infection and that under natural conditions an obligate relation exists similar to that demonstrated in *Calluna*.

5. Fungus invasion of the seedling tissues at germination and mycorrhiza formation are two distinct phenomena in *Vaccinium* as in *Calluna*. The former takes place regularly at an early stage of development; the latter is an annual event influenced largely by soil conditions.

6. Ovarial infection by mycelium has been observed also in *Vaccinium Vitis-Idaea*, L. *V. Myrtillus*, L., *V. pennsylvanicum*, Lam., *V. ovatum*, Pursh., *V. vacillans*, Kalm, and *V. corymbosum*, L. In view of this fact, the earlier observations of Stahl are shown to be erroneous and require revision. It is clearly impossible to raise seedlings of *Vaccinium Myrtillus* free from fungus infection ('völlig pilzfrei') by sowing seeds of this species in sterilized soil.

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EXPLANATION OF PLATE I.

Illustrating Dr. M. C. Rayner's paper on the Biology of Fungus Infection in *Vaccinium*.

Fig. 1. *Vaccinium corymbosum*. Seed from sound berry germinating under aseptic conditions on filter paper. Fine mycelium from seed invading tissues of emerging seedling. × 50.

Fig. 2. *V. Oxycoccus*. Longitudinal section of seed: *t.*, testa; *sp.*, endosperm; *e.*, space left by removal of section of embryo; *c.*, cavity at micropylar end. Droplets of oil from endosperm cells can be seen in various parts of the section. × 27.

Fig. 3. *V. macrocarpum*. Longitudinal section of fresh seed showing mycelium in and about outer cells of testa. Zeiss 8 mm. Obj., eyepiece no. 4.

Fig. 4. *V. Oxycoccus*. Longitudinal section of fresh seed: *t.*, testa; *p.*, pectose wall of outer layer of cells; *h.*, mycelium within cells of testa.

Fig. 5. *V. Oxycoccus*. Transverse section of young fruit showing fine mycelium in contact with developing seeds: *h.*, mycelium. Leitz Obj. 3, Zeiss eyepiece no. 18.

Fig. 6. *V. macrocarpum*. Longitudinal section of seed showing mycelium in cells of testa and traversing cell-walls. Zeiss Obj. 4 mm., eyepiece no. 6.

Fig. 7. *V. macrocarpum*. 'Pure culture' of seedlings from sterilized seed on organic nutrient agar. Slightly less than natural size.

Fig. 8. *V. macrocarpum*. 'Pure culture' of seedlings raised from endosperm and embryo only on organic nutrient agar. About half natural size.

Fig. 9. *V. macrocarpum*. Seedlings from aseptic culture similar to that shown in Fig. 8 arranged to show various stages of development. About natural size.

Fig. 10. *V. macrocarpum*. Seedlings raised from sterilized seeds growing in culture tubes of *Sphagnum*-extract agar. Two months from planting at three weeks after germination. Note adventitious roots from stem above surface of medium. Rather less than natural size.

Fig. 11. *V. macrocarpum*. Nearly median section of resting seed showing fine mycelium in endosperm tissue. Mounted Euparol. Zeiss Obj. 4 mm. eyepiece no. 6. Magnification as in Figs. 12, 13, 16.

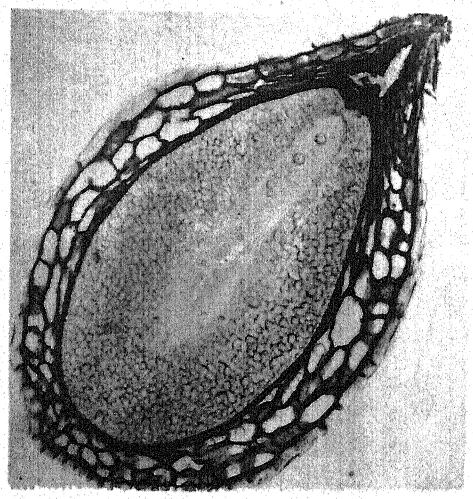
Figs. 12, 13. *V. macrocarpum*. Section through endosperm of 'pure culture' seedling during germination as shown in Fig. 8 after aseptic removal of seed coats, showing fine mycelium in cells of endosperm immediately in contact with embryo. Lactic-phenol. Zeiss Obj. 4 mm. eyepiece no. 6 (cf. Fig. 14).

Fig. 14. *V. macrocarpum*. Median sections through germinating seed from 'pure culture' as shown in Fig. 8. The photomicrographs in Figs. 11, 12, and 13 are from thinner sections through similar stages of seeds germinated aseptically after removal of seed coats. Zeiss Obj. a_{23}, eyepiece no. 6.

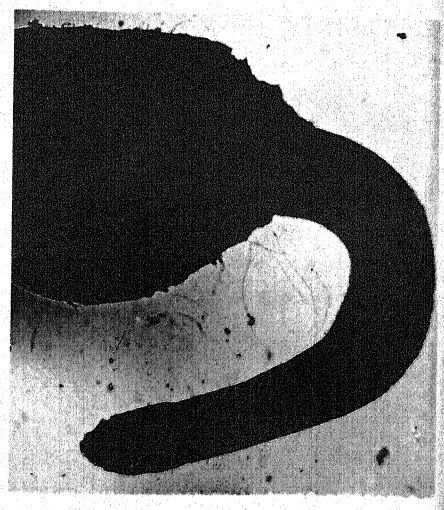
Fig. 15. *V. Oxycoccus*. Longitudinal section of resting seed showing fine mycelium in endosperm near cavity left by displacement of section of embryo. Stained gentian violet after lactic-phenol; mounted Euparol. Zeiss Obj. 4 mm., eyepiece no. 6.

Fig. 16. *V. macrocarpum*. Section through hypocotyl of one of the 'pure culture' seedlings shown in Fig. 9, showing fine mycelium in the cortical tissues. Mounted lactic-phenol. Zeiss Obj. 4 mm. eyepiece no. 6.

Fig. 17. *V. macrocarpum*. Plant raised in 'pure culture', as shown in Fig. 8, grown on for one year in sterilized peat watered with sterilized water. The roots showed typical sparse infection with fine mycelium. Reduced to less than $\frac{1}{4}$ natural size.



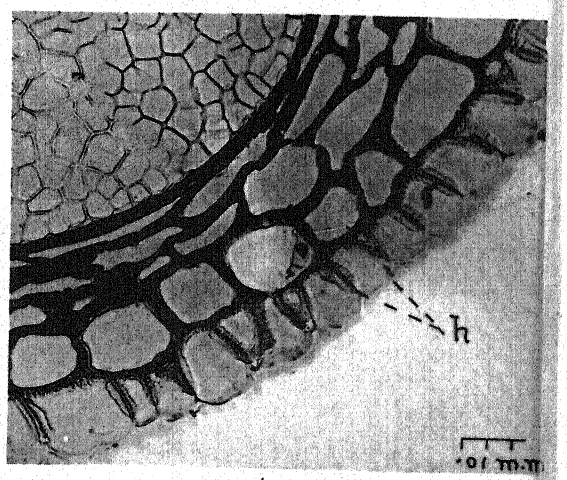
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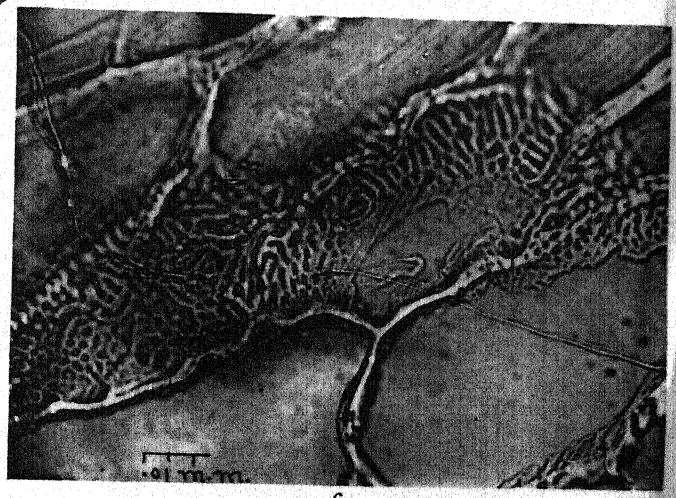
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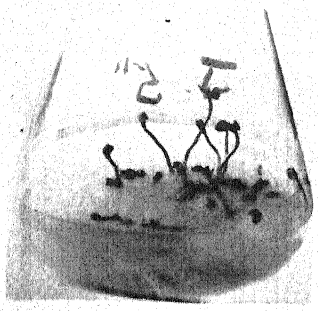


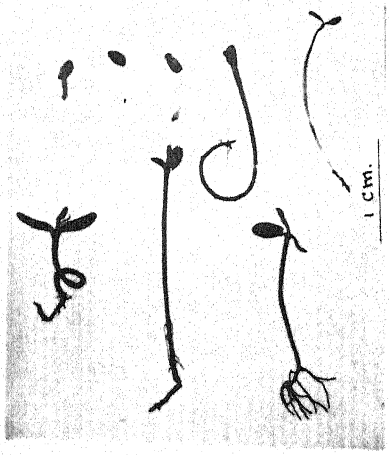
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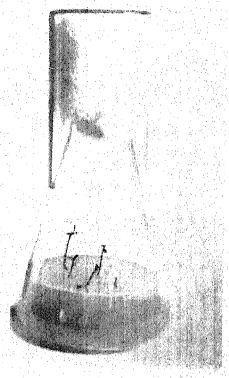


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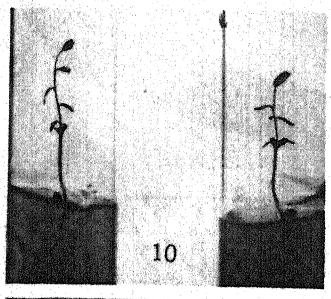
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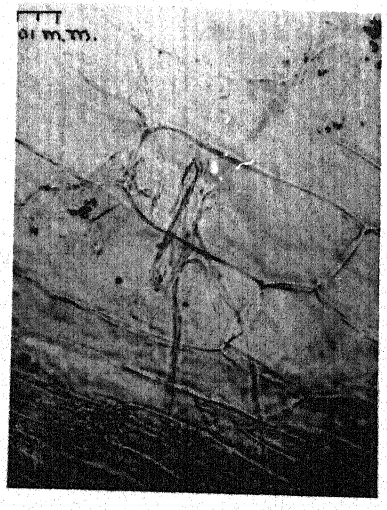




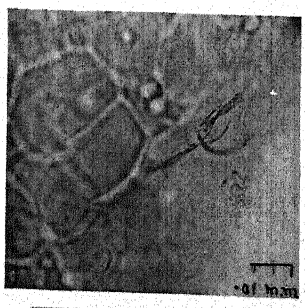
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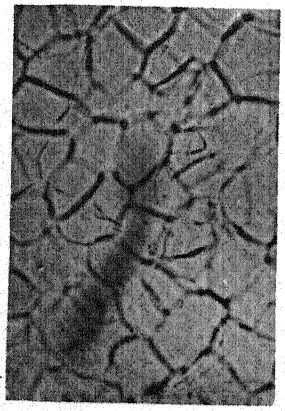
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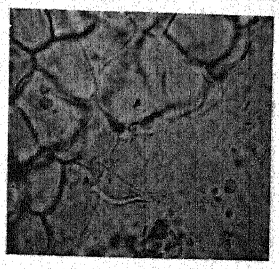
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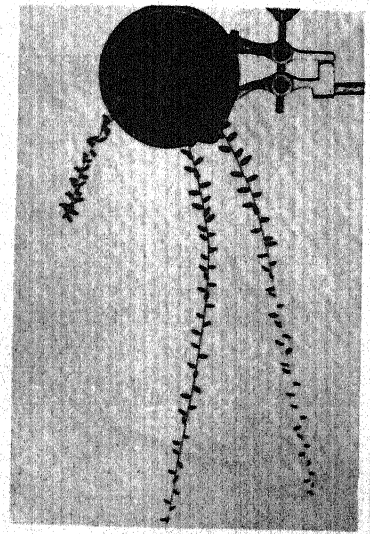
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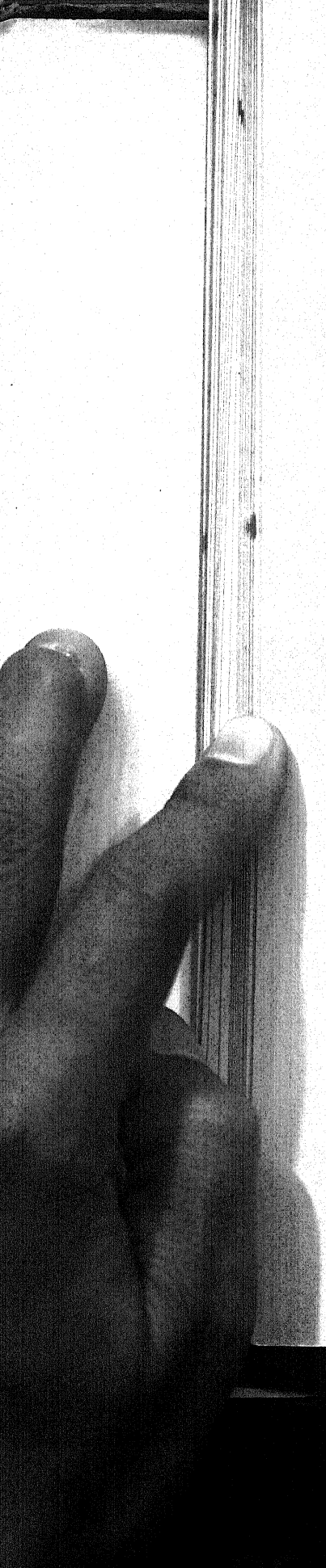
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A Method of Water Control for Sand Cultures.

BY

O. V. S. HEATH, A.R.C.S.

With two Figures in the Text.

INTRODUCTION.

IN work with sand culture where it is desired to keep the moisture content of the sand between fixed limits, the problem of water control presents certain difficulties. The method of weighing the complete culture at intervals and making up the loss in weight by adding water, involves a great deal of labour if the containers be large. On the other hand, if the containers be small, only very small plants must be grown so that the weight of the plant may be very low in relation to the weight of water in the sand. If this condition is not fulfilled, the increase in weight of the growing plant will cause the moisture content in the sand to decrease to too great an extent, since the total weight of the culture is kept constant by adding water. It is possible to allow for the increase in weight of the plant by taking samples of 'average plants' at intervals and finding their fresh weights. The 'average plants' being elected in each 'treatment' on the basis of such measurements as height and leaf area. This sampling of course involves a larger number of replications than would otherwise be necessary.

Another method which may be used for sand cultures is Livingston's 'auto-irrigator' (3). Water is supplied through a porous pot which is buried in the soil and connected to a reservoir at a lower level. Different moisture contents are produced by placing the reservoir at different distances below the porous pots. In unpublished work carried out by a previous worker at the Imperial College of Tropical Agriculture using this method for soil cultures of cotton, it was found that the roots of the plants grew round the porous pots in a thick mat and did not explore the soil at all. Another objection to the auto-irrigator is that it appears to provide very little aeration for the soil or sand. Water is supplied continuously through the porous pot, and there can be very little displacement of stale air from the pore-spaces such as would occur if periodic supplies of water were given at the top of the soil or sand.

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Kornev (1 and 2) carried out pot experiments using a method involving the same principle as does the auto-irrigator, and similar criticisms would appear to apply. He also devised an apparatus for measuring the 'absorbing power of soils', consisting of a porous pot filled with water and connected to a mercury manometer. He buried the pot in the soil, and found that the height to which the mercury rose before reaching equilibrium varied with 'the structure, degree of compactness, size of particles, and degree of moisture' of the soil used. He suggested that: 'the apparatus used may serve as a standard for the measurement of the soil moisture', though he apparently did not actually use it for this purpose. It was suggested to the writer by Dr. E. J. Maskell that such an apparatus would probably provide an efficient method of water control for sand cultures. A porous pot full of water and fitted with a manometer could be buried in each sand culture, and the manometers calibrated in terms of sand moisture content. The manometer reading would actually depend on the tension on the water films in the sand round the pot; but this tension must not be taken to be the force which root-cells would have to exert in order to take up water from the sand, as they would also be affected by the osmotic pressure of the nutrient solution. However, although the apparatus would not measure the 'water strain' upon the plant, it can be calibrated in terms of sand moisture content and used to show the volume of water to be added to a culture in order to bring the moisture content up to any desired value. Experimental work was accordingly undertaken to test the method.

It should perhaps be pointed out that with a manometer of narrow bore tubing, the volume of water which has to enter or leave the pot when the manometer reading changes is very small. The rate of passage of water through the pot is therefore relatively unimportant, whereas with the auto-irrigator it is extremely important.

Method.

Apparatus used. The porous pots used were Livingston porous porcelain candles, the most suitable type being 34·5 cm. long, and having an outside diameter of 4 cm. This type seemed the best as it was exactly the height of the kerosine tins used as containers, and would therefore tend to integrate the effects of the different moisture contents at all the different levels. Since only fifteen such candles were available it was necessary to use some short type candles also, which had been manufactured for use as atmometers and were varnished at the mouth; the length of the unvarnished portion was 8 cm. and the outside diameter 2·9 cm. It was found possible to use these short candles by fitting them all at the same level in the containers and by calibrating their manometers separately from those of the long candles.

The containers used were four gallon kerosine tins with the tops removed and coated inside with paraffin wax. The tops of the tins were covered with rubber-coated fabric which was tied down round the edge.

Sea sand was used, having been thoroughly washed and then dried and sieved. The particles were all less than 1 mm. in diameter, and all over 0·02 mm. diameter as determined by the sedimentation method. The maximum water-retaining capacity of the sand was 26·1 per cent. (Mean of three determinations, the standard error of the mean being 0·2).

Method of setting up and calibration. Preliminary experiments with porous candles, fitted with manometers and placed in sand at different moisture contents, showed that when equilibrium had been reached the manometer readings plotted against the sand moisture contents gave a reasonably smooth curve. Equilibrium was reached in about 24 hours from setting up, the delay being probably due to alterations taking place in the distribution of the water in the sand. Water would drain to the lower levels until distribution was such that the capillary forces at each level were just balanced by the weight of the 'water columns' below that level. For such a state of equilibrium to exist there would have to be smaller moisture contents near the top in order to give the larger capillary forces necessary to balance the longer water columns.

For reasons connected with the treatments in the sand culture experiment to be set up, twenty-five cultures were needed which made it necessary to use ten of the short type porous candles as well as the fifteen long ones. The types of manometer fitted to the long and short porous candles respectively are shown in Fig. 1. They were made with the two arms in contact in order to facilitate reading and reduce errors which would arise if the manometer were not quite vertical. Narrow glass tubing (inside diameter 3·5–4·0 mm.) was used.

The porous candles were boiled to remove air, and boiled water was used for filling them and the manometer tubes. The manometers were adjusted so that the mean level of the two mercury menisci would in all cases be the same distance (6·3 cm.) from the top of the container when the apparatus was set up. The corks had been coated with shellac to fill the pores and were sealed over with a molten mixture of pitch and shellac. Scales of graph paper (printed in 1 mm. squares) were stuck on to the manometers, the levels of the two mercury menisci being marked as zeros with the candles standing in water up to the level which the top of the tin would reach when the apparatus was set up. This gave a zero for the arm nearest the candle about 0·5 cm. lower than for the other arm, owing to the pressure of the 6·3 cm. of water in the tube between the level of the top of the tin and the mercury level. The object of thus marking the zeros with the candles in water was to provide that all the manometers should be in a standard condition of equilibrium. It also provided that zero readings

would be obtained with completely saturated sand (i.e. 26 per cent. moisture content) but, as will be seen below, the calibration curves did not tend towards zero at 26 per cent. owing to the effect of the column of water in the candle.

Each porous candle was set up in a tin with 26·3 kilogrammes of sand that had been mixed with a known volume of nutrient solution, the mixing

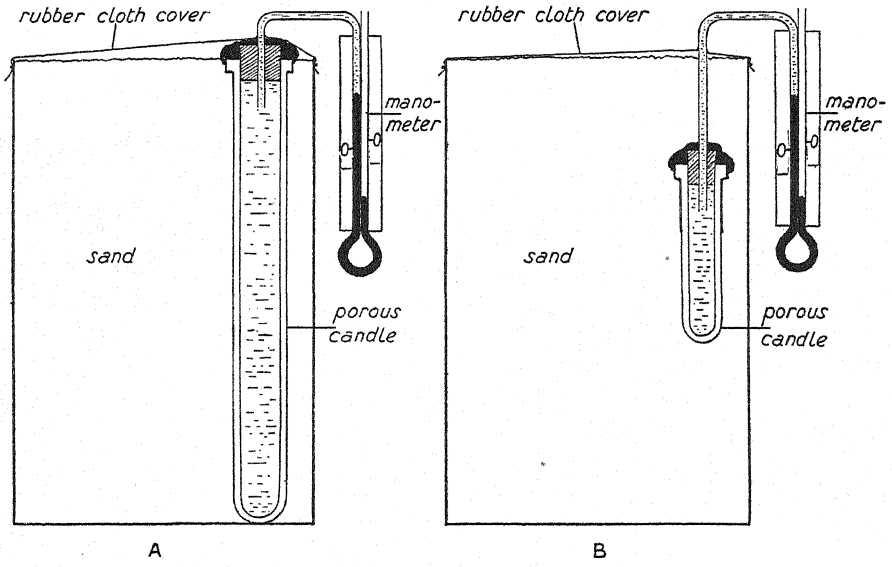


FIG. 1. Diagram showing 'set-up' of apparatus: A, long type candle; B, short type candle.

of the dry sand with the solution being done in four parts, a quarter of the moist sand being added to the tin at a time. Each quarter was well compacted, and two samples for moisture content determination were taken just before it was covered by the next quarter. The samples from the last quarter were taken immediately before the final tying down of the rubber cloth covering. The samples were lumped so as to give two composite samples A and D for each tin set up, one of the pair of samples from each quarter being placed in A and the other in B. The moisture contents of these composite samples were determined as percentages of the oven dry weight of sand and are shown in Tables I and II. The standard error of the mean of two samples were calculated from the fifteen pairs of determinations for tins with long porous candles, and also from the ten pairs of determinations for tins with short candles. For the former its value was 0·26, and for the latter 0·31.

Each porous candle was set up about 2 cm. away from the side of the tin, in the case of the short candles the unvarnished portion being midway between the top and the bottom of the tin. The slit in the rubber cloth for the manometer tube was sealed with adhesive plaster.

Manometer readings were taken soon after setting up and then at intervals up to 24 hours. After this the manometers were read daily for some days and any change was recorded. The readings are shown in Tables I and II.

TABLE I.

Long Porous Candles.

Moisture Contents from Samples.	Mean Moisture Content from Samples.	Manometer Readings.[1] (Times after Setting Up).			
%	%	5–25 min.	1–3 hrs.	24–30 hrs.	Later Readings.
4·1 4·1	4·10	6·05 cm.	6·05 cm.	6·50 cm.	8·05 cm.
4·33 4·35	4·34	5·75 „	5·75 „	6·75 „	7·15 „
5·80 5·76	5·78	5·25 „	5·20 „	5·30 „	5·35 „
5·95 5·76	5·86	5·30 „	5·30 „	5·45 „	5·45 „
7·1 7·2	7·15	4·55 „	—	4·80 „	4·95 „
7·4 7·3	7·35	4·50 „	4·60 „	4·85 „	4·85 „
9·4 9·4	9·40	3·85 „	—	4·25 „	4·25 „
9·66 9·69	9·68	4·20 „	—	4·35 „	4·35 „
11·17 12·06	11·62	3·70 „	3·85 „	3·85 „	3·90 „
12·07 11·61	11·84	3·85 „	3·95 „	3·95 „	3·95 „
12·23 12·02	12·13	3·70 „	3·80 „	3·90 „	3·90 „
13·3 13·3	13·30	3·45 „	3·55 „	3·60 „	3·60 „
13·9 13·3	13·60	3·60 „	3·60 „	3·70 „	3·70 „
15·92 15·24	15·58	3·25 „	3·25 „	3·30 „	3·30 „
16·51 15·01	15·76	3·40 „	3·40 „	3·50 „	3·50 „

Equilibrium was very nearly, if not quite, reached in 24 hours in all cases, except the two manometers attached to long porous candles in sand with 4 per cent. moisture. With these two manometers a considerable further rise took place (see Table I). As, however, these high readings gave points right off the curve, and as the manometer of the short candle in sand with 4 per cent. moisture had reached equilibrium in 24 hours, it was thought better to take the 24-hour readings for calibration.

[1] Each reading is the sum of the rise of one meniscus and the fall of the other, each from its own zero mark.

TABLE II.

Short Porous Candles.

Moisture Contents from Samples.	Mean Moisture Content from Samples.	Manometer Readings.[1] (Times after setting up).			
		5–25 min.	1–3 hrs.	24–30 hrs.	Later Readings.
%	%				
4·28 4·33	4·31	5·50 cm.	5·80 cm.	6·15 cm.	6·20 cm.
5·31 5·85	5·58	5·05 ,,	5·10 ,,	5·35 ,,	5·45 ,,
5·74 5·73	5·74	5·10 ,,	5·10 ,,	5·10 ,,	5·10 ,,
6·3 7·3	6·80	4·65 ,,	—	4·70 ,,	4·70 ,,
7·3 7·0	7·15	4·60 ,,	—	4·75 ,,	4·85 ,,
9·60 9·49	9·55	4·20 ,,	4·30 ,,	4·35 ,,	4·35 ,,
9·55 9·64	9·60	4·20 ,,	4·15 ,,	4·20 ,,	4·20 ,,
9·5 11·1	10·30	3·85 ,,	3·90 ,,	3·90 ,,	3·90 ,,
13·6 14·0	13·80	3·45 ,,	3·45 ,,	3·45 ,,	3·45 ,,
13·9 13·7	13·80	3·50 ,,	3·50 ,,	3·55 ,,	3·55 ,,

Using the 24-hour readings of the manometers (y) and the moisture contents found by sampling (x), curves were plotted for the long and short porous candles respectively. These curves were apparently portions of rectangular hyperbolae, since plotting x against xy gave an approximation to a straight line in each case. Curves were therefore fitted to the data by the least square method, using the full equation for a rectangular hyperbola, viz.,

$$(x+a)\ (y+b) = k.$$

For the long porous candles the best fit obtained was the graph of the function

$$x = \frac{44{\cdot}71}{y-1{\cdot}12} - 4{\cdot}305$$

Using this equation values of x were calculated for the observed values of y, and the deviation of each observed value of x from the corresponding calculated value was taken. These deviations were squared and the standard deviation calculated from

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-3}} = \sqrt{\frac{\Sigma d^2}{12}}$$

[1] Each reading is the sum of the rise of one meniscus and the fall of the other, each from its own zero mark.

The value of σ was found to be 0·635 and allowing for the variance due to sampling in the determination of moisture contents this becomes 0·578 (i.e. 5·9 per cent. of the mean of the observed values of x).

For the short porous candles the best fit was obtained using the equation

$$x = \frac{61{\cdot}55}{y - 0{\cdot}533} - 7{\cdot}243$$

The standard deviation was calculated in the same way as for the long porous candles (but in this case $n = 10$ so $\sigma = \sqrt{\frac{\Sigma d^2}{7}}$) and was found to be 0·614. Allowing as before for the variance due to sampling this becomes 0·529 which is 6·1 per cent. of the mean of the observed values of x.

The calculated curves and the points plotted from the data are shown in Fig. 2, p. 78. Taking the values from the calculated curves, tables were made for the long and short porous candles giving moisture contents corresponding to different manometer readings.

The moisture contents as measured by this method are significant to about 1·2 per cent.[1] of the weight of sand. It should, however, be remembered that when the same porous candle and manometer were being used, as in finding the water uptake of a plant growing in one of the cultures, the error would be much less than this as the standard deviation was found using different manometers. In addition it should be noted that, with a plant transpiring at a reasonable rate, the water uptake could be measured with some accuracy by adding water daily to bring the moisture content back to the same value and working with the total volume of water added per week. The reason for this is of course that the errors are not cumulative but tend to average out.

It will be seen that the calibration curves (Fig. 2) do not tend towards zero at 26 per cent. moisture content. For 26·1 per cent. moisture content the manometer readings calculated from the equations are 2·6 cm. and 2·4 cm. for the long and short candles respectively. This is largely, if not entirely, due to the pull which the column of water in the candle exerts when the latter is not surrounded by free water. The effective length of this column of course alters with the rise or fall of the mercury by an amount equal to that rise or fall, and this must affect the shape of the curve to a slight extent. It seems probable that a slightly better fitting curve might be obtained if this factor were eliminated, and as a possible refinement of the method the manometer could be so adjustable that the mercury meniscus in the tube nearest the candle could always be made level with the bottom of the candle at the time of reading. This would eliminate all effect of the water column in the candle since it would always be balanced by the water in the tube above the mercury. Any other fixed level might be used

[1] This deviation would only be exceeded by chance once in twenty times.

instead of the level of the bottom of the candle, in which case the effect of the water column in the candle, though not eliminated, would be constant and would not affect the shape of the curve. It is of course necessary that there should be no free water at the bottom of the container as this would alter the effective length of the water column in the porous candle, and it should be noted that in the calibration none of the moisture contents used was high enough to give free water at the bottom.

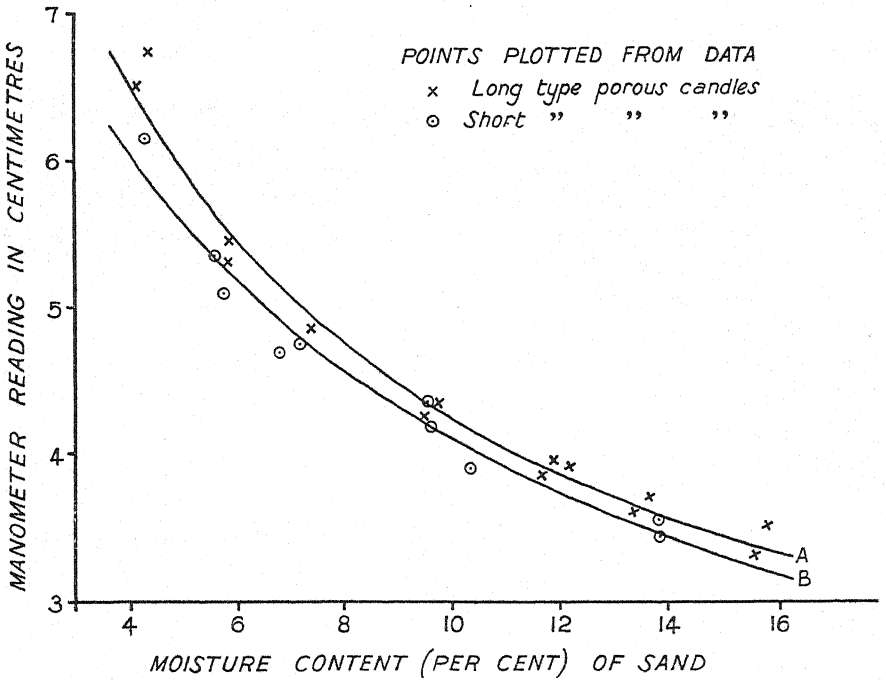


FIG. 2. Calculation curves: A, large type candle; B, short type candle.

The method in use. After the calibration had been completed, the tins of sand with their porous candles and manometers were used for a sand culture experiment with cotton. The correct volumes of water were added to bring the moisture contents in some of the tins up to 8 per cent., and in others 16 per cent. In all cases the new position of equilibrium was reached in from 5 to 15 minutes. Cotton seedlings growing in sand were transplanted into the tins. Most of the cotton plants failed to make good growth owing to a curious physiological affection of the leaves, which may have been due to an unsuitable nutrient solution. The water control apparatus was, however, extremely satisfactory throughout the season. The manometers reached the new position of equilibrium rapidly on the addition of water to the sand, and the new readings were consistent with the volumes of water added. On washing out the roots of the plants at the end

of the experiment, no tendency to grow round the candles rather than elsewhere in the sand was noticeable, either in the case of large well-grown plants or small ones.

It seems likely that the method might be used for soil cultures if suitable precautions were observed as to type and uniformity of soil.

Summary.

The construction and calibration of an apparatus for measuring the moisture contents of sand cultures is described. This apparatus consists of a porous porcelain candle full of water, buried in the sand, and with a mercury manometer attached. By calibrating the manometers in terms of sand moisture content, it is possible to measure the latter significantly to 1·2 per cent. of the weight of sand. If the apparatus is used for measuring the water uptake by a plant growing in sand culture the accuracy must be much greater. It is suggested that this apparatus provides a satisfactory method of water control for sand cultures.

In conclusion, I wish to thank Professor Cheesman for his unfailing assistance and criticism. I am also deeply indebted to Dr. E. J. Maskell for very many invaluable suggestions and criticisms. This work was carried out while holding a Senior Studentship from the Empire Cotton Growing Corporation.

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The Life-history of Sorosphaera radicale, sp. nov.

BY

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AND

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With Plate II.

CONTINUING our investigation of the root fungi from the ponds and marshes in the neighbourhood of Sevenoaks, we were fortunate to find a considerable quantity of the fungus which is the subject of this paper. The fungus, although resembling the other members of the Plasmodiophorales in many respects, exhibited certain features which seemed to bear upon the phylogeny of the genus *Sorosphaera*, and it was thought desirable that a study should be made of the life-history of this new species.

Sorosphaera radicale is found in the roots of a number of different grasses growing in the marshes and damp meadows in the neighbourhood of Dunton Green, Kent. It has also been found along the banks of a large lake between East Grinstead and Crawley, Sussex. No apparent effect is produced upon the host which would enable diseased plants to be detected, except a slight reddening of the stem and leaf bases. The fungus only attacks the root-hairs which swell up very considerably as a result.

Material from both localities was collected and brought into the laboratory in a living condition. The roots were examined, and those which appeared likely to be attacked were fixed for about twelve hours in Bouin's Fluid. The individual roots were then examined under a microscope, and the diseased ones were put aside and stained in Heidenhain's haematoxylin and mounted in balsam. The method employed was similar to that previously described (4). Additional care, however, was necessary in order not to injure the delicate root-hairs, and for the same reason the stain was not removed to the same extent with acid alcohol, as the acid appeared to affect the walls of the root-hair.

Observations were also made upon living material in the hope of gaining some idea of the method of spore germination which is still only

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imperfectly known in any of the group. The results were far from successful. Since no empty spore masses were found, it seems probable that the 'sorosphere' is liberated *en masse* from the root-hair when this ceases to function, and consequently it was not altogether surprising that the spores could not be artificially induced to germinate. Careful study of the roots mounted in water adhering to them, however, enabled us to study the structure of small zoospores which were occasionally found in numbers around the infected roots; they were small oval or spherical structures with a single apical flagellum, which vibrated rapidly and was only made out with difficulty. The flagellum was about twice the length of the zoospore. Some of these zoospores were observed to enter the root-hairs, but it was difficult to be sure that the root-hairs entered had not been previously damaged, since they appeared to find no difficulty in gaining entrance, such as would be expected if the cell-wall had to be penetrated. These zoospores, however, bear a close resemblance to those previously observed in *Ligniera junci* (4), and those described by other workers in the genera *Spongospora* (10) and *Plasmodiophora* (6).

As other species of the Plasmodiophorales have also been collected from the meadows near Dunton Green, it was felt that it would be difficult to be certain that all the early stages found in these roots were caused by *S. radicale*. *L. junci* was originally described under the name of *S. graminis* from grass collected from the same locality. All the early stages were therefore worked out, using material collected from the other locality where, as far as is known, no *L. junci* is to be found.

It was found that the fungus passes the whole of its life-cycle within the root-hairs and does not enter the cells of the root, although occasionally it may be found far up the root-hair in the epidermal cell from which it originated, giving a superficial appearance of living in the root tissue.

The plasmodium resembles in general features that already described in *L. junci*, only the finer structure of the protoplasm enabled it to be distinguished. The plasmodia vary considerably in size, but they do not usually completely fill the root-hair. In their early stages they do not cause any hypertrophy, but as the plasmodium grows the root-hair swells out until, finally, it is as much as three or four times its normal diameter. Although not invariable, it is generally found that the swelling is restricted to that part of the root-hair in which the fungus is lying; the part nearest the epidermis is frequently quite normal, but if the centre part of the hair is swollen the tip is only rarely unaffected. It has been previously pointed out (2) that there are many reasons for considering that the life-history of the Plasmodiophorales is a short one; this is particularly the case in this species, since the spore masses have to be produced before the root-hair decays.

It was not found convenient to study the nuclear division in any detail,

the nuclei are smaller than those of either *L. junci* or *S. veronicae*, resembling in size more closely those of *Plasmodiophora brassicae*, nor was this necessary, since we now know the characteristic type of division which is common to all the species. At the same time, since the type of the nuclear division is so characteristic of the group, it was necessary to find sufficient stages to make it certain that the general method of division was similar. This was successfully done, though the divisions obtained were not so clear as those in *L. junci*. They were enough, however, to indicate that there was no divergence from the general scheme.

The life-history appears to be closely similar to that of *L. junci* or *S. veronicae*. The zoospore enters the root-hair, and there grows at the expense of the food taken in by the host plant. The original nucleus divides by the protomitotic method characteristic of the Plasmodiophorales and a plasmodium is formed. This increases in size, and before maturity may contain over a hundred nuclei. A wall is now secreted around the plasmodium, and the whole mass passes into a sporing stage. The nuclear divisions at this point are difficult to make out, but by comparison with what has been observed in other species it is concluded that a reduction takes place, and that the nuclei which are present in the spores contain the single or haploid number of chromosomes.

It is the structure of the spore mass which renders this species easily distinguished from any previously described. In *L. junci* and *L. verrucosa* which have been known to attack grass roots the spores are not arranged in any definite system, though at times they do appear to be grouped in a more or less spherical mass. In the genus *Sorosphaera*, of which only *S. veronicae* has been described, the spores are arranged in a hollow sphere. In the new species *S. radicale* the same system is adopted, only that instead of always being in spherical they are more commonly found in elongated oval or ellipsoidal masses. These masses are cemented together by a definite membrane, as can be sometimes observed in *S. veronicae*, moreover, these masses are hollow. This feature is clearly brought out in the two photo-micrographs (Pl. II, Figs. 4 and 5), which show the same spore mass in surface view and in median section. This spore mass is very characteristic when observed in living material since it is yellow brown in colour, and shows up clearly in unstained preparations. It was not found possible to determine the nature of this colouring matter, but owing to the irony nature of the soil in which it was growing and the fact that many of the roots themselves are darkly stained with iron, it seems likely that the colour of the spores is due to the same substance—iron oxide. Tests with ammonium hydroxide showed a greenish precipitate, but it was difficult to determine with certainty whether the precipitate was only produced in the cell contents, or whether it was restricted to the wall of the spore mass. For the same reason other colour tests were only of doubtful value.

It was noticed that these spore masses were only found in the older root-hairs, and this, together with the absence of any direct evidence regarding spore germination or dispersal, lead to the conclusion that the whole 'sorosphere' is dropped off when the root-hair is lost. Although careful search was made, no empty masses were found in any of the root-hairs. Very little information is available regarding the time which root-hairs function in the case of grasses, but since they are only developed for a short distance from the growing-point, it may be concluded that they do not remain for very long, grass roots grow at a fairly rapid pace, especially those of the annual species. No evidence is available as to precisely when a root-hair is sufficiently developed to receive the zoospores, but young plasmodia have been found in root-hairs which have not attained more than a third their normal length.

It may, therefore, be concluded that from the time the zoospore enters the root-hair to the time when the 'sorosphere' is mature is comparatively short. It is quite likely that the time is shorter in this species than in some of the other genera, and this may account for the great scarcity of any nuclear divisions in the preparations examined.

A comparison of the size of the individual spores with those of *S. veronicae* show that they are smaller. The average of a number of independent measurements indicate that the diameter of the spore is 3 μ. The complete 'sorospheres' vary very much in size; the smallest observed were the spherical ones, which measured about 20 μ in diameter; the largest were the elongated oval masses, which were found up to 57 μ long by 16 μ wide. In a few cases masses were found in which the central part was smaller than at the two ends, giving the appearance of the early stage of invagination of a gastrula. It was very difficult to estimate the number of spores in any one 'sorosphere', but in an average sized one it was calculated that some 500 spores were present. A comparison with *S. veronicae* shows that the number of spores in *S. radicale* is considerably greater.

There is much clearer evidence of a common membrane around the spore mass in *S. radicale* than can be made out in *S. veronicae*, though it is probable that this feature is common to both species.

Discussion.

The genus *Sorosphaera* was made by Schröter in 1886 (12) to include the species *S. veronicae*, which he had previously referred to the Ustilaginales. Later, in 1895, Rostrup (11) found this species in *V. hederifolia* in Denmark, and thought that there were hyphae among the spore balls and replaced the fungus in the Ustilaginales. In 1904 Trotter (16) discovered the organism in *V. arvensis* and came to the conclusion that it was not a member of the Myxomycetes, although he was doubtful if it should

be classed in the Ustilaginales. In 1909 Maire and Tison (8) studied the organism in much greater detail, and showed both by cytology and in the life-history that *S. veronicae* was a member of the Archimycetes and placed it in the Plasmodiophorales. The following year Blomfield and Schwartz (1) discovered and also described the life-history and cytology from material found in this country. These two descriptions agree very closely in all essential features, and they have been further confirmed by observations made more recently on fresh material.

In 1910 the life-history of another species, *S. junci*, was described from the roots of Juncus (13). This species differed considerably from *S. veronicae*, however, as it caused little or no hypertrophy, and the spores were not arranged in a very definite system. In 1911 Maire and Tison (9) rightly transferred *S. junci* to a new genus *Ligniera*. Subsequent work on that genus (14) has shown that the other species of *Sorosphaera*, *S. graminis* and also those later described in the genus *Ligniera* (15), are only host varieties of the one species *L. junci*.

Although since that date further members of the Plasmodiophorales have been described, none have been placed in the genus *Sorosphaera*.

In 1925 Fron and Gaillat (7) described a species under the name of *L. pilorum*, which caused hypertrophy of the root-hairs of species of grass. In the swollen character of the root-hairs this is similar to what we find in *S. radicale*, but from the illustrations which accompany that paper it is clear that the spores are not arranged in a definite 'sorosphere'. It has been pointed out previously (2) that *L. junci* occasionally forms its spores in scattered masses in swollen root-hairs, but that this is an abnormal rather than a normal state, and it is considered that *L. pilorum* represents a form of *L. junci*. We have paid special attention to the question of whether there could be any relation between *L. junci* and *S. radicale* through *L. pilorum*, and are quite satisfied that such does not exist, but that *S. radicale* is a new and distinct species.

One other possibility presented itself, both *S. veronicae* and *L. junci* have been found in the locality at Dunton Green, where this new species was observed, and it seemed possible that *S. radicale* might be a hybrid between these two, since it exhibited some of the characters of each genus. When, however, the fungus was found at another locality in Sussex this view became untenable since here, though a careful search was made, neither *L. junci* nor *S. veronicae* was found.

S. radicale shows in its vegetative stages the typical life-history of a member of the Plasmodiophorales, and it is difficult to distinguish its plasmodium from that of *L. junci*. The stages in spore formation are precisely similar to those of *S. veronicae*, the aggregation of the amoebulae being unlike that found in *L. junci*, where no such aggregation takes place.

In a recent paper (5) the relationships of the various members of the

Plasmodiophorales was discussed, and it was pointed out that there was little in the life-histories to indicate what was the primitive and what the more advanced condition. This new species seems to indicate that there is a definite relationship between the genus *Sorosphaera* and the genus *Ligniera*, and that hypertrophy is not a feature upon which too much importance must be based. Despite the comparatively close affinity between the two genera, it is a matter of very great difficulty to determine which of the two should be considered the more primitive. If, as has been pointed out before, spore systems are considered more advanced than scattered spores then *Sorosphaera* has been evolved from *Ligniera*; and, moreover, there is evidence that a reduction in the number of spores is usually a specialized feature.

In order to show the remarkable similarity in the spore masses between the two species of *Sorosphaera* photomicrographs were taken under the same magnification both from the surface of the 'sorosphere' and also of the same mass in median section (Pl. II, Figs. 4–8).

In order to clear up the position of the genus *Sorosphaera* and the two species included in it, the Latin diagnoses are appended.

SOROSPHAERA (Schröt) (after Saccardo).

Sporae ellipsoides-cuneatae, plurime parietibus lateralibus in soros cavos arcte coniugatae. Intra cellulas plantarum nutricum nidulantur.

S. veronicae (Schröt.) = *Tuburcina veronicae* (Schröt.), olim.

Pulvere sporarum cinnamomea; soris sporarum globosis vel ellipsoideis 18–28 μ × 18–22 μ; in cellulis parenchymaticis, valde incrassatis, numerose nidulantibus; sporis ovoideis, cuneiformibus, apice applanatis, cuticula communi tectis, parietibus lateralibus coniunctis et sorum cavum formantibus; sporis 8–9 μ × 4–4·5 μ episporio levi, pallide brummeo, apice crassiori; tuberculis cylindraceis, saepe curvulis 3 mm. crassis.

Hab. in caulibus et petiolis *Veronicae hederifoliae*, et *V. triphyllae* in Silesia; in *V. arvensis* et *V. Chamaedrys* in Gallia; in *V. Chamaedrys* in Britannia.

S. radicale, sp. nov.

Soris sporarum globosis vel ellipsoideis cavis et membrana una amplexis. Soro sporarum vel amoeba solo iacente in pilo tumido radicis. Diam. sporarum 3 μ; diam. sororum 20–57 μ × 16–20 μ.

Hab. in pilis radicis graminum diversorum ad Dunton Green, Kent, et ad Crawley, Sussex in Britannia.

SUMMARY.

1. A new species of the genus *Sorosphaera* has been discovered. Its life-history has been followed, and has been found to agree closely with that of the other members of the Plasmodiophorales.

2. Its cytology has not been worked out in detail, but it agrees in all essentials with that characteristic of the Plasmodiophorales. The nuclear divisions during the growth of the plasmodium are protomitotic, while those prior to spore formation are apparently meiotic. The akaryote stage, although undoubtedly present, was difficult to make out.

3. A review of the literature relative to the genus *Sorosphaera* is made, and special reference is given to the only other member of the genus, *S. veronicae*. The relationship of the new species to the genus *Sorosphaera* and also to the genus *Ligniera* is discussed.

4. The Latin diagnoses of the genus *Sorosphaera* and the two existing species is appended.

The microscopic apparatus used in this investigation was obtained through a grant from the Dixon Fund of the University of London, for which we wish to record our thanks.

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EXPLANATION OF PLATE II.

Illustrating Dr. W. R. I. Cook and Dr. E. J. Schwartz's paper on *Sorosphaera*.

All figures from photomicrographs.

Fig. 1. *Sorosphaera radicale*. A young plasmodium lying in a swollen root-hair. × 230.

Fig. 2. *Sorosphaera radicale*. A plasmodium about to form a spore mass. Note the swollen character of the root-hair. × 230.

Fig. 3. *Sorosphaera radicale*. A typical spore mass with the associated parts of the root-hair, showing the position of the swelling relative to the epidermis of the root. × 230.

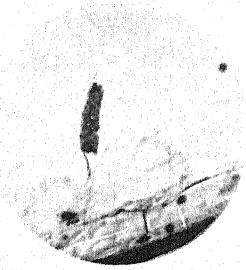
Fig. 4. *Sorosphaera radicale*. A large spore mass showing the appearance in surface view. Note the hexagonal structure of the individual spores. × 550.

Fig. 5. *Sorosphaera radicale*. The same spore mass as in Fig. 4 in median section, showing the character of the 'sorosphere'. × 550.

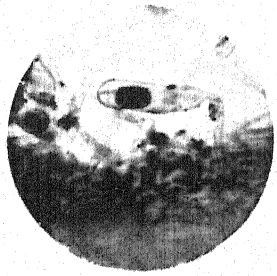
Fig. 6. *Sorosphaera radicale*. An uncommon spherical mass of spore more closely resembling that found in *S. veronicae*. × 550.

Fig. 7. *Sorosphaera veronicae*. A typical spore mass in surface view or comparison with Fig. 4. × 550.

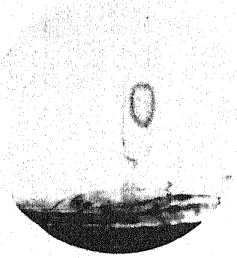
Fig. 8. *Sorosphaera veronicae*. The same spore mass as in Fig. 7 in median section for comparison with Fig. 5. × 550.



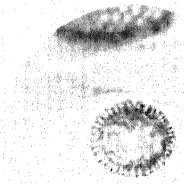
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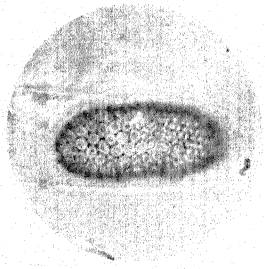
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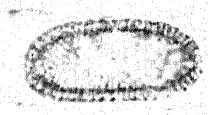
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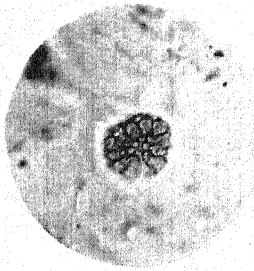
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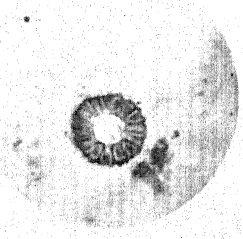
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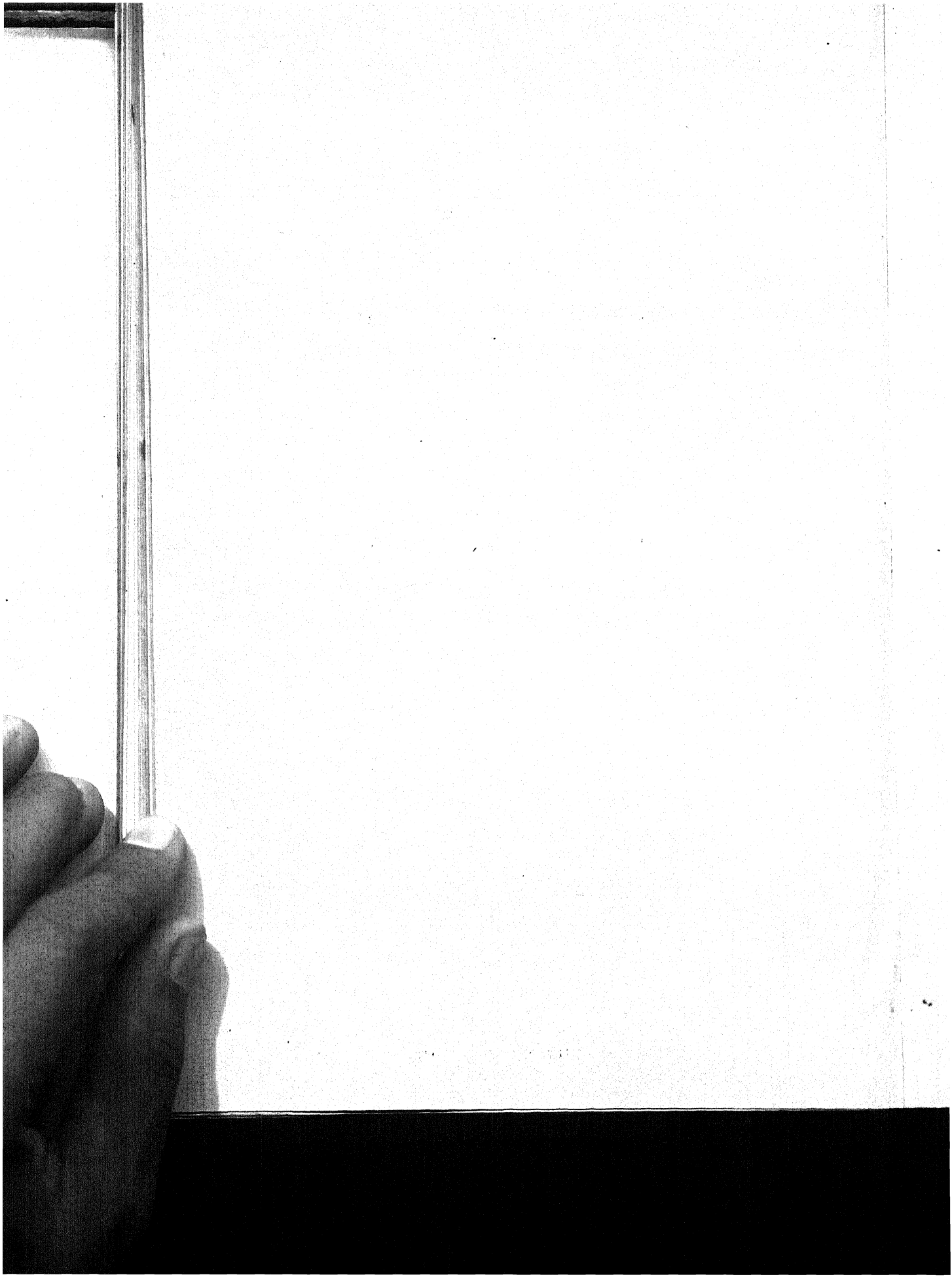
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W.R.I.C. photo. Huth, coll.

COOK & SCHWARTZ — SOROSPHAERA.



The Phosphate Requirement of Barley at Different Periods of Growth.

BY

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(*Rothamsted Experimental Station*).

With Plate III and nine Figures in the Text.

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THE growing realization of the importance of phosphorus in plant nutrition has led to a good deal of attention being focused on the periods of the life-history in which the element is of the greatest value to the plant. The question of economy in phosphate utilization and supply has a very direct bearing on agricultural practice, and the experiment here to be considered has been undertaken with this in mind. On arable land phosphatic manures are generally applied before or at the time of sowing, to ensure that they are properly mixed with the soil, and the soundness of this procedure as regards plant nutrition receives further support from the results here presented.

SCHEME OF EXPERIMENT.

With a view to testing the effect of alteration in the phosphate supply at different growth periods, experiments were made in which barley plants were grown for varying times in solutions containing phosphate, and were then transferred to other solutions minus phosphate. Vice versa, plants

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grown without phosphate at first ultimately completed their growth in the presence of a full supply of phosphate, the successive transfers of five plants each being made in both cases at fortnightly intervals. This gave two series of plants grown respectively with and without phosphate for 0, 2, 4, 6, 8, 10, 12, 14, and 16 weeks, then receiving the reverse treatment. Simultaneously two series of five plants each, started with and without phosphate respectively, were changed into the reverse solution at the end of every fortnight, giving a double set which only had phosphate available for half-time, evenly distributed throughout the growth period. It was hoped that this might indicate whether the temporary presence or absence of phosphate was more critical at any one point in the life-history than at another. At the time of each transfer five similar plants from both original sets ($\pm$ *P*) were determined for dry matter and phosphate content, giving a measure of the position when each change of treatment occurred. The plants were grown singly in bottles of 600 c.c. capacity, and the solutions were changed weekly. The roots of the plants undergoing transfer were carefully washed to remove as much of the old solution as possible, to avoid carrying over more phosphate than could be avoided, the no phosphate roots being washed for the sake of uniformity.

The solutions used were as follows:

	With Phosphorus.	Without Phosphorus.
Potassium nitrate	1 grm.	1 grm.
Potassium di-hydrogen phosphate	0·3 ,,	—
Potassium mono-hydrogen phosphate	0·27 ,,	—
Sodium chloride	0·5 ,,	0·5 ,,
Magnesium sulphate	0·5 ,,	0·5 ,,
Calcium sulphate	0·5 ,,	0·5 ,,
Potassium chloride	—	0·4 ,,
Ferric chloride	0·04 ,,	0·04 ,,
Water	to make up 1 litre (pH 6·2)	to make up 1 litre (pH corrected to 6·2 by the use of NaOH solution)

The complete nutrient solution supplied phosphorus at the rate of 116·5 parts per million, equivalent to 266·7 p.p.m. P_2O_5. Analyses made of average plants at fortnightly intervals showed a maximum uptake of 0·1646 grm. P_2O_5 during two weeks of active growth with two changes of solution. This represents 0·0823 grm. per week, equivalent to 82·3 p.p.m. P_2O_5 taken up from a solution originally containing 226·7 p.p.m. Thus it is clear that an ample reserve of phosphate was always available for use throughout the periods that phosphorus was supplied, and no question of deficiency effect due to inadequate rationing of phosphorus can therefore arise.

A pure strain of Goldthorpe barley was used, graded between 0·04 and and 0·05 grm. weight, sown in sawdust April 1st, 1927, and put into nutritive solutions April 8th. The seed coats showed a tendency to develop mould, and as soon as the endosperm was absorbed they were removed and

no further trouble occurred from that source. Week by week the number of tillers on each plant was counted, each new tiller being labelled by number. Towards the end of the experiment many tillers died, and the progress of this decrease was also recorded.

The plants that bore ears were harvested as they became ripe, when all green colour had disappeared from grain and awns, cutting being spread over three weeks. Even at the end of that time a few of the tiny plants grown without phosphate were still green, but nothing would have been gained by keeping them longer. Most of the plants without ears were cut eight days after the beginning of harvest, in a succulent green condition, as they tended to die off in a decayed state if left too long.

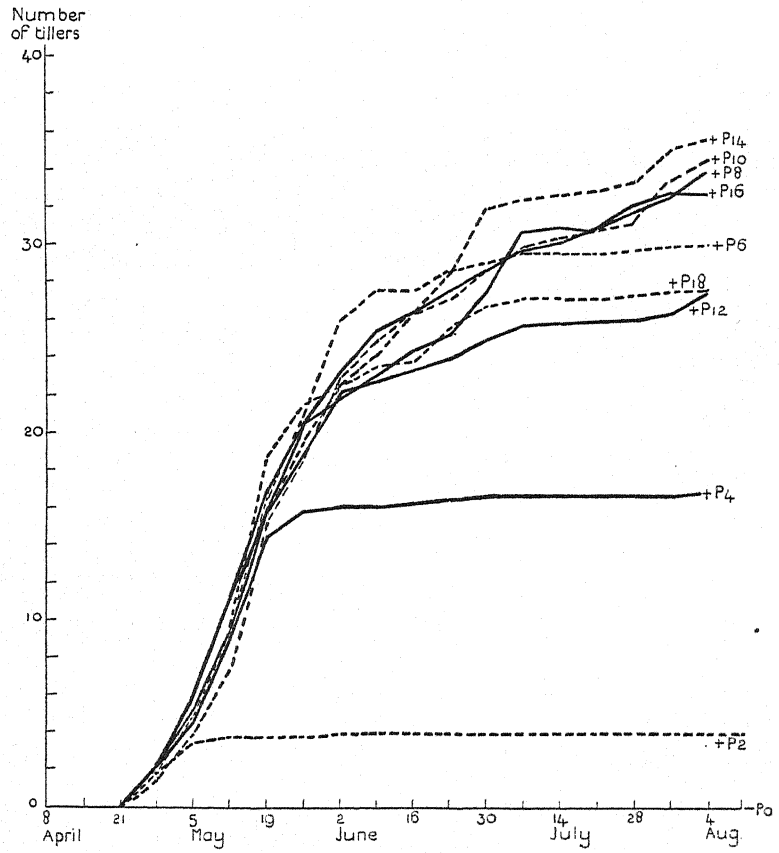
At the time of harvest each earing tiller was measured from the base to the middle of the ear, and the number of grains and sterile flowers from each ear was counted. Shoot, leaves, root, grain and sterile flowers were weighed separately, and to the leaves and shoots were added those which had died during growth and been kept till required. The dry weights only were recorded, and phosphate determinations were made on grains and sterile flowers together, shoot and leaves together, and on the roots. The latter presented difficulty, as it was impossible to be certain that all the phosphate from the nutritive solution was removed from the root in spite of most careful and prolonged washing in many changes of water when harvested. When the analytical figures became available, however, it was obvious that the washing had been more complete and successful than was hoped for. (Data given in Appendix A.)

Tiller Production.

When phosphate was *present* at the outset tiller formation began from two to three weeks after the seedlings were put into solutions and proceeded at a rapid rate, an average of twenty tillers per plant appearing in the next four weeks, after which the rate began to slacken off. During the first period all the plants were very uniform in behaviour, as the total range of variation for groups of five plants did not exceed three tillers (Text-fig. 1, May 26th). During the later period the majority of new tillers were small and insignificant, and tended to die off without developing much. Individual plants varied greatly in this respect, causing considerable divergence in the tiller numbers for groups of five plants. This divergence set in weeks before any change of treatment occurred in those plants which grew with phosphate for twelve to sixteen weeks, and therefore was not connected in any way with the removal factor. Considering the curves for all treatments, it therefore seems probable that there is little or no significant difference in the total number of tillers produced, provided phosphorus is available during the first six weeks. If it is only present for four weeks, tiller formation continues for another fortnight as rapidly as where phosphorus is still

available, but then is suddenly checked for all time. If the removal occurs after a fortnight three or four tillers are produced during the next two weeks, and then formation ceases except in isolated cases.

When phosphate was *withheld* for the first fortnight, the number of tillers produced was slightly depressed for about two months, after which

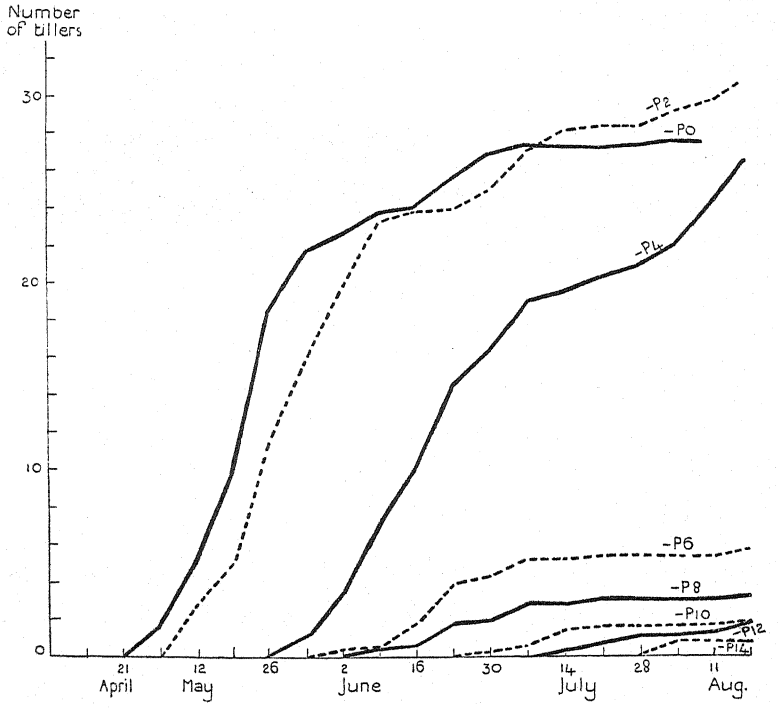


TEXT-FIG. 1. Graph showing total number of tillers formed in barley grown with phosphate at first, then without phosphate. ($+P_2$, &c., indicate number of weeks with phosphate.)

there was no significant difference from the plants which had received phosphate all through until towards harvest time, when the initially deprived plants were still forming new tillers (Text-fig. 2). After four weeks without phosphorus, tiller production proceeded at as rapid a rate as in the above case, but showed hardly any slackening at harvest, by which time about as many tillers were present as in the fully-fed plants. Longer periods of deprivation did not admit of so marked a recovery, a few tillers being formed when phosphorus was first supplied, after which the impetus seemed to cease. The energy of tiller production decreased as the period

without phosphate became longer, plants grown for sixteen weeks without phosphorus failing to show any response whatever, though some might still have been shown if the plants had been kept longer.

In both cases, whether phosphate was initially supplied or withheld, the critical period seemed to lie between the first four and six weeks, a definite change in the direction of the effect of the treatment occurring between



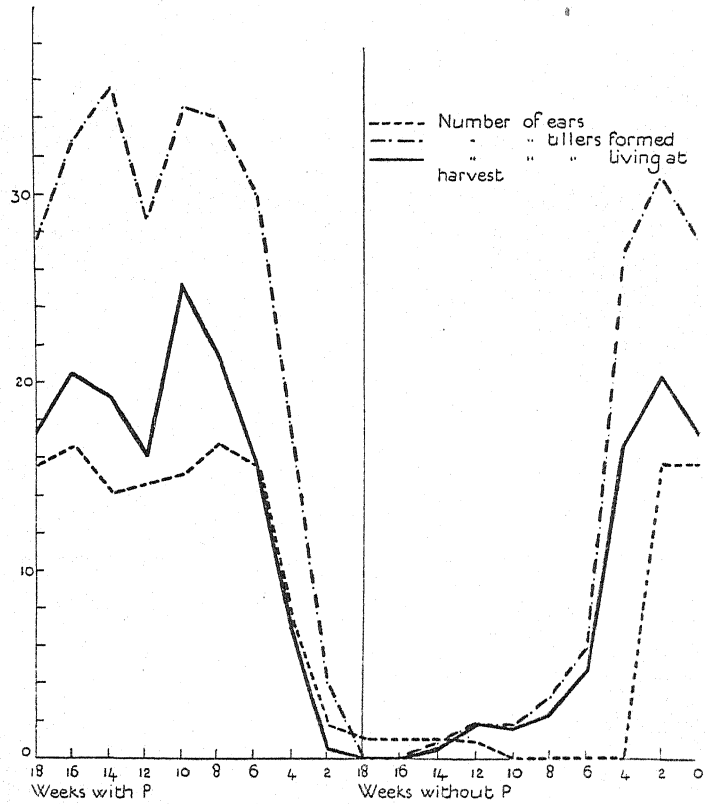
TEXT-FIG. 2. Graph showing total number of tillers formed in barley grown without phosphate at first, then with phosphate. ($-P_2$ &c, indicate number of weeks without phosphate.)

these dates. The considerable length of time that plants starved of phosphorus retain their power of responding by tiller production when phosphorus is given is worthy of note.

EAR FORMATION.

Provided phosphate was *available* for the first six weeks, removal at any later date did not significantly affect the number of ears produced, which amounted, roughly speaking, to about one-half the total number of tillers formed (Text-fig. 3). With shorter periods in phosphate ear production declined rapidly, the same ratio to total tiller formation being maintained approximately. The majority of the ears were produced by the earliest tillers, the reduction in tiller numbers being mainly due to the death

of those formed later in life. In some cases, however, where phosphate was presented for a limited time at the beginning there was a tendency for one or more of the very first tillers to fail to produce ears. The reduction in the number of ears was accompanied by a reduction in size and quality, and those ears with only two weeks in phosphate tended to die off without emerging properly, before the grains had filled out. Despite variation in



TEXT-FIG. 3. Graph showing correlation between (*a*) total number of tillers formed; (*b*) number of living tillers at harvest; (*c*) number of ears produced in barley, as affected by varying phosphate treatment.

size, all the plants started with phosphate were of similar type, showing erect tillers and ears, though leaf production was more prolific with some treatments, as will be shown later (Pl. III, Fig. 1).

When phosphate was *omitted* during the early history a different course of events resulted. Omission for the first two weeks had no adverse effect, but omission for four weeks changed the character of the plant completely and entirely inhibited ear formation (Pl. III, Fig. 2). The number of tillers ultimately formed was much the same in both cases, but with four weeks omission of phosphate they were very sappy and flabby in nature, readily

attacked by mildew, and altogether unhealthy and abnormal in type. The large number of tillers and absence of ears gave the plants a very curious and characteristic appearance. As the time without phosphate increased to ten weeks the type of growth remained the same, but the number of tillers fell rapidly and the plants became very small. With twelve weeks and more without phosphate the growth type again changed, the succulence of the few tillers (if any) disappeared, and the thin feeble main shoot in most cases developed a small badly-formed ear, with very few grains, of which most were sterile. The need of phosphate very early in the life-history is clearly shown, and is evidently associated primarily with ear formation. Its absence at the critical moment abruptly checked this, but did not at the same time affect tiller production, thus accounting for the large bunchy plants produced after four weeks without phosphate. Longer periods of deprivation reduced tiller production until a point was reached when a characteristic 'no phosphate' type was fixed, in which the energy of the plant was directed to the formation of a travesty of an ear at the expense of the phosphorus stored in the seed, little or no attempt at tiller formation being made.

Grain Formation.

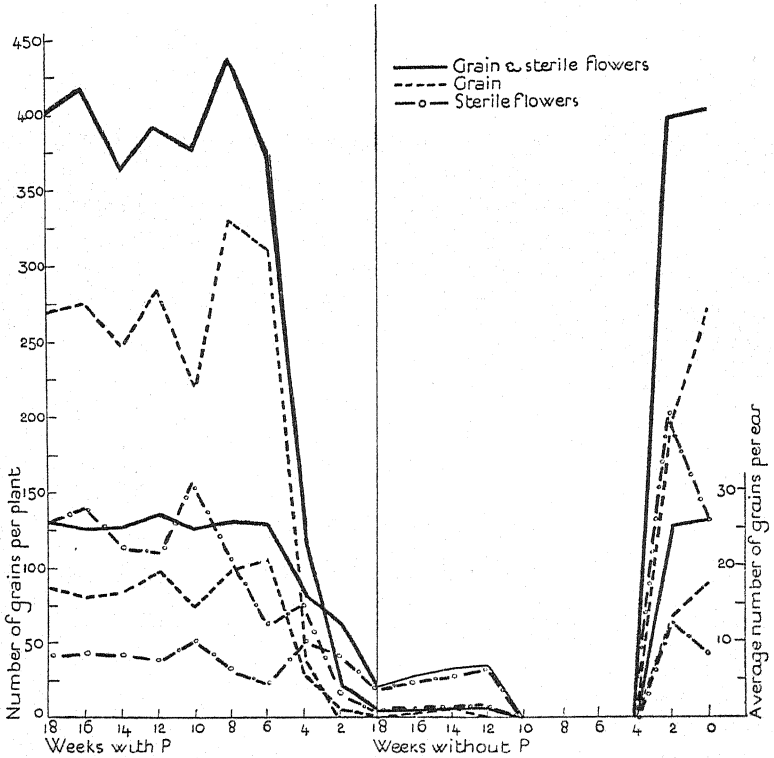
Provided phosphate was present for the first six weeks, little significant difference occurred in the total number of grains produced if it were withheld at any later date (Text-fig. 4). The largest proportion of grains to sterile flowers occurred with six weeks phosphate, the proportion being less with increased phosphate provision, though there was no regularity in the variation of the ratio with different periods of time. When phosphorus was only available for the first four weeks or less the total number of grains fell below that of the sterile flowers, until with entire absence of phosphorus no grains at all were produced.

The course of events for the average number of grains per ear was very similar to the above, showing that the decrease in the number of ears due to acute phosphate deficiency (Text-fig. 3) was paralleled by a similar reduction in grain formation per ear. The close similarity in the average number of grains per ear provided phosphate was given for six weeks or longer, was noteworthy, the maximum variation being two grains per ear.

The omission of phosphate at the outset was of far greater significance to grain formation than its removal at any time after initial supply. The absence of phosphorus for the first fortnight did not affect the total number of flowers produced, but it so decreased the grains and increased the sterile flowers that the proportions of the two classes were equal instead of being in the ratio of 2/1 as when phosphorus was supplied throughout. As already noticed, ear and grain formation were entirely inhibited with longer periods of phosphate omission up to twelve weeks, at which point feeble single ears

were produced with a larger proportion of sterile flowers to grains. The actual number of grains and sterile flowers per ear tended to decrease somewhat with lengthened periods of phosphate omission, being least where no phosphate was supplied at any time.

It is evident from these results that the critical period of need of phosphate for grain formation is at the beginning of development and not at the



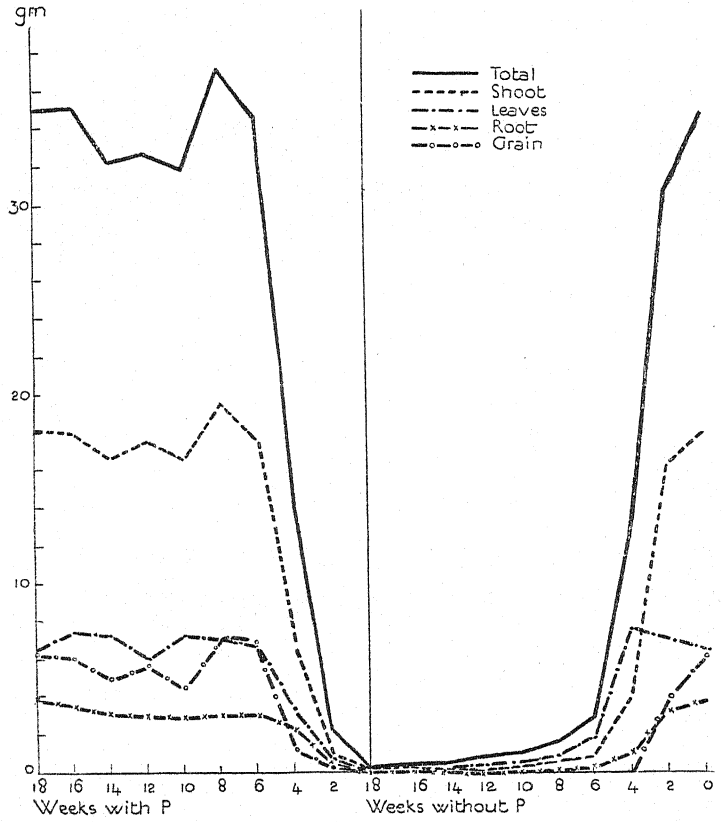
TEXT-FIG. 4. Graph showing effect of varying phosphate treatment upon (*a*) total number of grains and sterile flowers per plant; (*b*) average number of grains and sterile flowers per ear.

end, the necessity being most marked between the second and fourth weeks, and fully supplied by the sixth, under the particular environmental conditions of the experiment. Even the first fortnight is of significance, as provision for that period alone tripled the flower production per ear compared to that when no phosphorus was supplied (twelve to four), whereas omission for the same period influenced development and reduced the proportion of grains to sterile flowers (from two: one, to one: one).

DRY WEIGHT.

The dry weights of the whole plant, and of its component parts, were not significantly affected *provided phosphate was supplied* for the first six

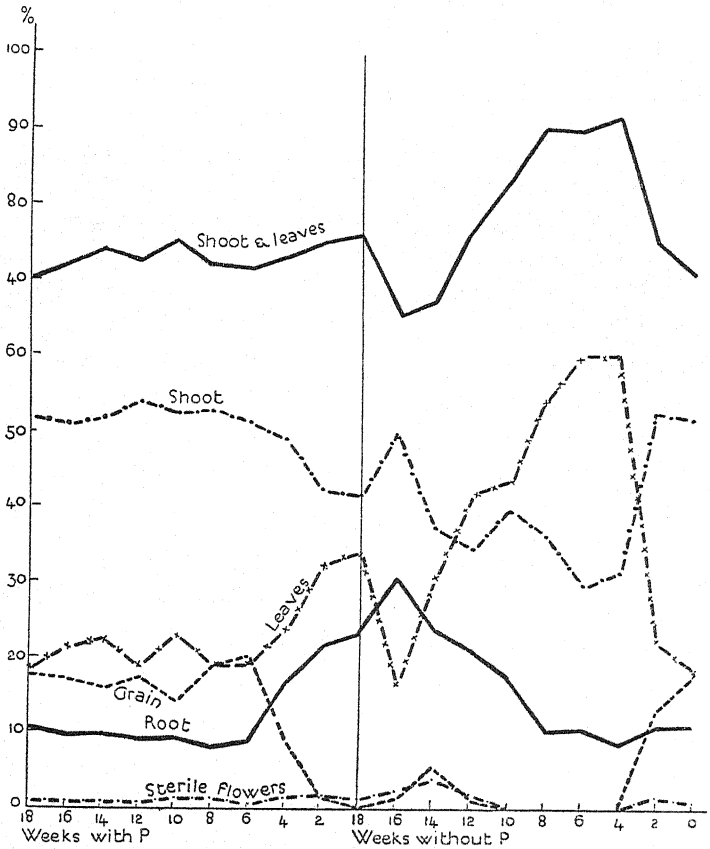
weeks or longer, unless the heavier weight of grain and shoot with the eight weeks' period should prove to be real after further experiments. Shorter treatment with phosphate caused a sharp depression of dry weight, specially marked in the shoot and grain, less evident in the leaves and root (Text-fig. 5). The proportion of each component to the total dry weight was unaffected down to the six weeks period, but the reactions varied with



TEXT-FIG. 5. Graph showing actual dry weight of barley and its component parts as affected by varying phosphate treatment.

shorter times of phosphate provision. The percentage of the total dry weight provided by the grain declined very rapidly under these conditions, the shoot showed a definite but less marked decrease, whereas the leaves and root increased steadily in proportion as the time of phosphate supply was reduced to nil (Text-fig. 6). This latter phenomenon is always very obvious when the plants are handled during growth. The proportion of stem and leaf together, however, is very little affected by the time of initial exposure to phosphate, and the actual variation in proportion lies between the root and grain in such cases of phosphate deficiency.

The *omission of phosphate* even for the first fortnight caused some decrease in the dry weight of all parts of the plant except the leaves, and deprivation for a month accentuated the fall, but still left the leaves unaffected (Text-fig. 5). The slight rise shown is unlikely to be significant. The marked decline of the various components continued with six weeks

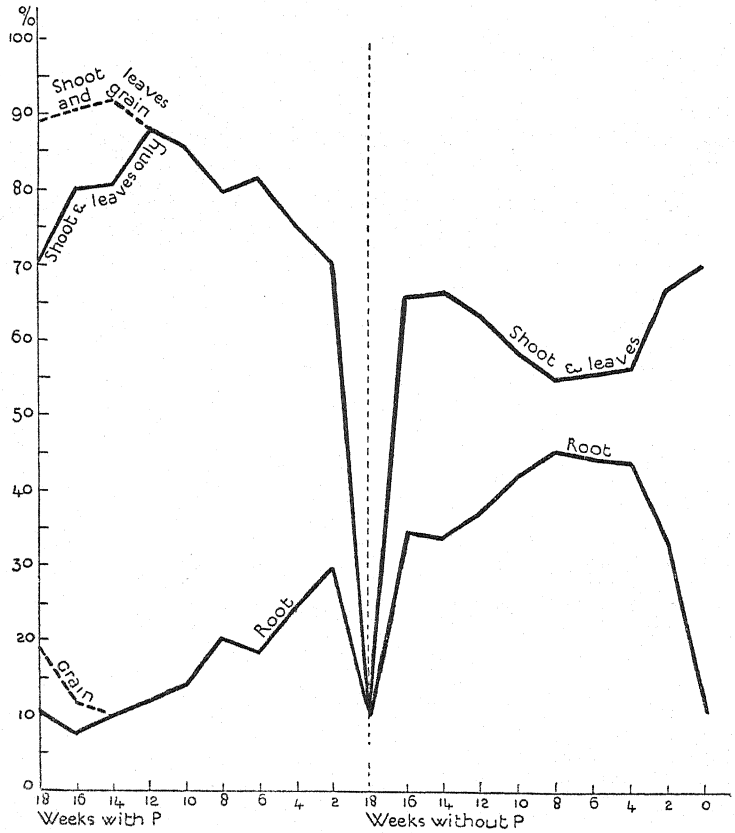


TEXT-FIG. 6. Graph showing effect of varying phosphate treatment on the percentage proportion contributed by the different parts of the barley plant to the total dry weight.

absence of phosphate, and at this time the leaves were adversely affected to the same degree. After this the dry weights continued to decrease slowly with longer periods without phosphate, the total weight being only 0·356 grm. in the entire absence of phosphate supply. A striking effect of the initial omission of phosphate is that the proportion of leaf is higher than that of stem for the intermediate periods (Text-fig. 6), the difference being very great with from four to eight weeks' omission and decreasing with longer periods, until with fourteen weeks' starvation the normal order reasserts itself and the stems outweigh the leaves. The proportion of root to total shoot

is little affected until after eight weeks' phosphate omission, when it rises rapidly and steadily with the increase of time of starvation.

Under normal conditions of nutrition the dry weight of the entire shoot (stem, leaves, and grain) increases in proportion, at the expense of the root, till a few weeks before harvest, in this case forming ultimately about 90 per cent. of the total (Text-fig. 7). This is also the case if phosphate is pro-



TEXT-FIG. 7. Graph showing percentage proportion contributed by different parts of the barley plant to total dry weight at different periods of growth.

vided for six weeks or longer, otherwise the root becomes proportionately heavier as the initial time of phosphate supply is lessened (Text-fig. 6). When phosphate is not available at the beginning, the ratio of shoot to root is throughout less than in the latter case. As the period of omission increases, the proportion of root rises rapidly at first, but after about eight weeks' omission, when the type of growth is changing (see p. 95) root development becomes less rapid than that of the shoot, the difference being the more marked the longer the period of initial phosphorus starvation. This variation again emphasizes the importance of the first few weeks in the

phosphate nutrition of the plant, the whole course of the life-history being influenced by the conditions at that stage far more than by changes at later dates.

Phosphate Uptake.

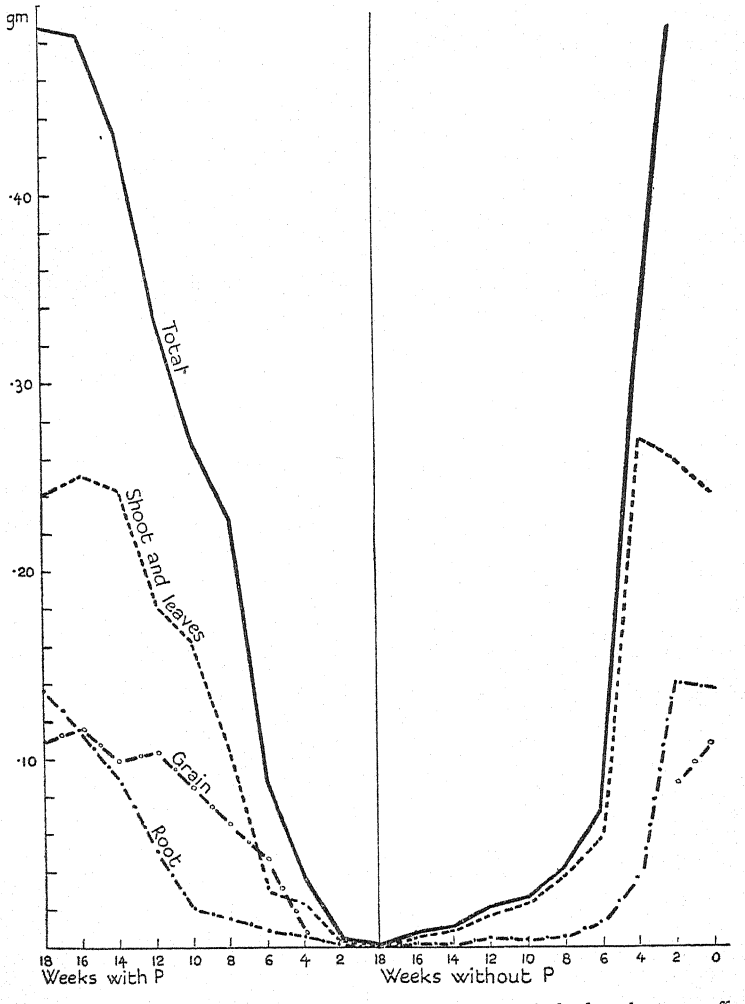
(a) *Phosphate present at beginning.*

Very little phosphorus was absorbed when phosphate was presented during the first fortnight only, but with longer periods of initial presentation the uptake increased rapidly, practically in direct proportion to the length of time phosphate was available in the solution (Text-fig. 8). A few weeks before harvest, however, the uptake at first slackened slightly, and then entirely ceased during the later stages of ripening of the grain, the presence of phosphate in the solution at this time being therefore redundant. Phosphate uptake under these conditions corresponds closely to that recently shown by Arrhenius (1) for barley grown in water cultures and analysed for phosphate at fortnightly intervals. It is very significant that sufficient phosphate was absorbed in the first six weeks to enable the plant to make its maximum amount of dry matter, the necessary percentage of P_2O_5 amounting to 0·25 per cent. of the ultimate dry matter formed. The further supplies of phosphate absorbed during later periods had no influence on dry matter production, merely increasing the percentage of phosphate present. This is a further indication of the importance of phosphate supplies in the early history of the plant, the later supplies having little effect upon yield, though they improve the quality of the crop as far as nutritive value is concerned. The general trend of this result corresponds with that obtained by Pember (5), who states that during the first three weeks of growth in culture solutions his barley plants absorbed enough P_2O_5 to produce plants maturing nearly the maximum amount of seed. Pember and McLean (6) also indicate the very small amount of phosphate necessary to produce a good yield, 0·2 per cent. P_2O_5 in dry matter being adequate.

Relation between Dry Weight and Phosphate Uptake.

+P Weeks.	Dry Weight Total.	P_2O_5 Total.
	grm.	grm.
0	0·36	0·0006
2	2·39	0·0039
4	14·08	0·0361
6	34·62	0·0878
8	37·16	0·2275
10	31·80	0·2700
12	32·62	0·3373
14	32·29	0·4321
16	35·16	0·4825
18	35·02	0·4871

The distribution of phosphate to the different parts of the plant varied considerably at different periods. In the *shoot* (including leaves) the percentage of P_2O_5 was constant for periods of phosphate presentation up to

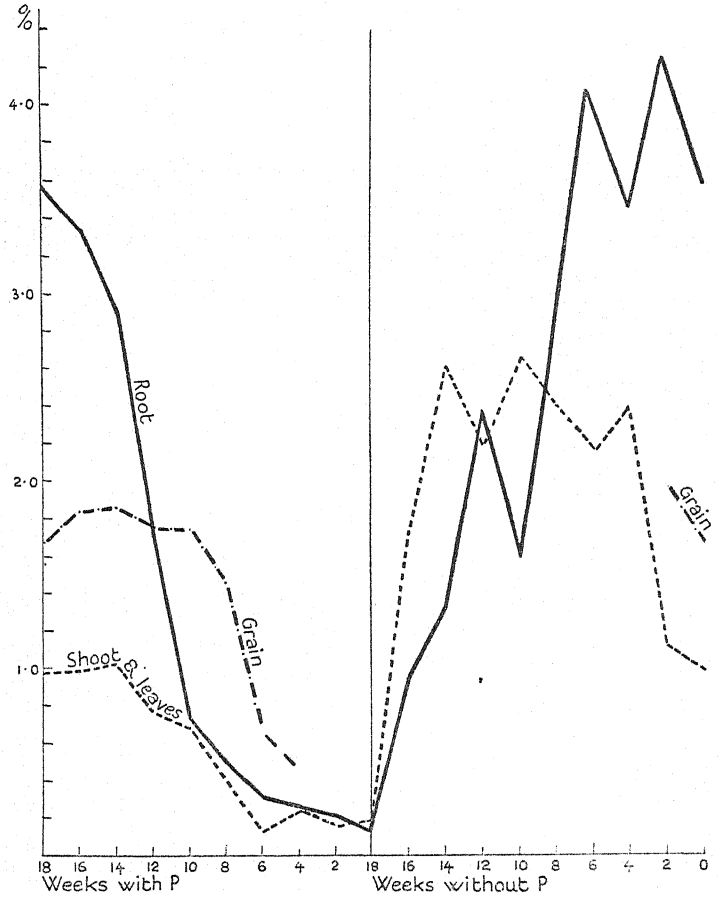


TEXT-FIG. 8. Graph showing actual phosphoric acid present in barley plants, as affected by varying phosphate treatment.

six weeks, after this the percentage increased steadily until about a month before harvest, and then again remained constant (Text-fig. 9). This variation in percentage corresponds with a relatively slow P_2O_5 reception for the first six weeks presentation, followed by a rapid increase in quantity, until at a month before harvest the supply was abruptly cut off.

In the *grain* the percentage of phosphate increased rapidly until the initial time of presentation reached ten weeks, but with longer periods of

availability the percentage remained fairly steady. The greater part of the phosphate had passed into the grain after the first twelve weeks, the addition during the last six weeks being small. This compares well with the result obtained in barley grown under field conditions (2). In unmanured



TEXT-FIG. 9. Graph showing percentage of P_2O_5 in dry matter of barley as affected by varying phosphate treatment.

soil the phosphate uptake of the whole plant ceased five weeks before harvest, and migration into the grain ceased about ten days later, whereas in manured soil the uptake continued rather longer, but ceased in the same way some time before harvest.

The phosphate uptake of the *root* was distinctly different from that of the rest of the plant. The P_2O_5 percentage remained low at first, rising slowly up to ten weeks' initial presentation of phosphate, then proceeded much more rapidly to fourteen weeks' initial phosphate, after which the rate

slackened again. With more than twelve weeks' initial phosphate the percentage of P_2O_5 in the root was considerably greater than in either the shoot or grain. The actual amount of phosphate in the roots was very small till the change with the ten weeks' set, but then rose rapidly and steadily until the end. It seems unlikely that the increase is due to extra phosphate remaining adherent to the surface of the root and not removed by washing, as during the time (ranging up to eight weeks) that the plants were grown

Total P_2O_5 Present in Barley.

A. Plants transferred to solutions without phosphate at periods indicated.
B. Plants harvested at periods indicated.

After Weeks with Phosphate.	Shoot.		Root.		Grain.		Total.	
	A.	B.	A.	B.	A.	B.	A.	B.
	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
2	0·0029	—?	0·0010	—?	—	—	0·0039	0·0014
4	0·0236	0·0051	0·0058	0·0074	0·0068	—	0·0361	0·0125
6	0·0317	0·0431	0·0095	0·0213	0·0467	—	0·0878	0·0644
8	0·1041	0·1154	0·0152	0·0601	0·1082	—	0·2275	0·1755
10	0·1626	0·2017	0·0218	0·0473	0·0855	—	0·2700	0·2490
12	0·1823	0·2952	0·0515	0·1184	0·1035	—	0·3373	0·4136
14	0·2439	0·3445	0·0907	0·1449	0·0976	0·0453	0·4321	0·5347
16	0·2510	0·3306	0·1154	0·1138	0·1161	0·0413	0·4825	0·4857
18	0·2409	0·2409	0·1367	0·1367	0·1095	0·1095	0·4871	0·4871

in solutions without phosphate, after their initial periods with phosphate, it may be expected that any adherent phosphate would be absorbed or washed off into the solutions, which were changed weekly. The indications are, therefore, that phosphate absorption from the solution continued to a very late date, only ceasing shortly before harvest. Earlier than this, however, migration into the shoot slackened and finally ceased, the excess phosphate remaining perforce in the root, instead of being distributed throughout the plant, as occurred earlier in the life-history. The question arises as to whether loss of phosphate from the plants took place during the periods during which they were growing in no phosphate solution. A comparison of the calculated amounts of P_2O_5 present in these plants with others harvested at each fortnightly interval provides no definite evidence that this was the case, possibly until the plants were approaching maturity. For each of the earlier periods (2, 4, 6, 8, 10 weeks' initial phosphate) actually *less* P_2O_5 was present in the plants sampled than in those grown on without phosphate, the difference obviously being covered by the variation in individual plants and the experimental error. For the next two or three periods the position was reversed, and it is possible that here some actual loss was incurred by the natural dying off of parts of the root at this stage of growth. The P_2O_5 content of the root at the actual time of change was in general higher than at harvest time after growing in no phosphate solution, probably on account of phosphorus being transferred from the roots to

other parts when starvation occurred. This did not occur in the last two or three weeks when, as indicated before, migration within the plant has apparently ceased. The grain is obviously a first charge on the plant in the matter of phosphate supply, as the full complement was passed into the grain even when the plant received phosphate for the first eight weeks only. This stability was attained at the expense of the shoot, whose phosphate content at harvest was consistently lower than that of the samples taken at time of transfer to no phosphate solutions.

(b) *Phosphate absent at beginning.*

Phosphate deficiency in the early stages of growth had a drastic influence upon the uptake of phosphate, paralleled closely by its effect upon the dry weight. An extremely rapid drop occurred when phosphate was withheld from two to six weeks at the beginning, after which a more gradual decrease set in with lengthening periods of phosphate privation (Text-fig. 8). There was nothing here to parallel the high dry matter production with small phosphate uptake as occurred in the reverse series (see (a)).

With regard to the distribution throughout the plant, the bulk of the P_2O_5 throughout was in the shoot. The first rapid drop in phosphate content in the root occurred after four weeks' initial starvation, a fortnight less than was needed to induce a similar drop in the shoot, indicating that the shoot is somewhat more resistant than the root in this respect. The percentages of phosphate in the shoot and root followed markedly different courses. In the shoot a very heavy percentage was present after periods of starvation ranging from four to fourteen weeks, suggesting a considerable intake when phosphate was eventually supplied, without, however, sufficient vitality or response on the part of the plant to enable it to utilize this phosphate in the production of additional dry matter (Text-fig. 9). In the root, on the contrary, the percentage of phosphate remained at a level much above that in the shoot, approximating to that of a normally fed plant until after six weeks' initial phosphorus starvation, and then suddenly and rapidly decreased more or less in proportion to the increase in the time phosphate was withheld, falling below the percentage in the shoot at this stage.

Pember's results are in striking contrast to these, as he found that after serious semi-starvation of phosphorus during the early stages barley was able to utilize abundant later supplies satisfactorily, storing much of it in the seed. The crux of the discrepancy would appear to lie in the different effect of *total* and *semi*-starvation in the early periods, but comparative experiments on this point are still wanting.

Discussion.

Under the conditions of the experiment, in which phosphate was either entirely withheld or supplied in ample quantity for certain periods, it is

evident that it is during the early stages of growth that the presence or absence of phosphate is of most vital importance. When a sufficiency of phosphate was supplied for the first few weeks tillering, ear and grain formation and the production of dry matter proceeded as well and to the same degree as though phosphate had been present throughout. The uptake of phosphorus, however, did not follow the same course, but continued steadily until shortly before harvest, so that the percentage of phosphate present in the dry matter formed was correlated with the length of time that phosphate had been available in the solution. Similar conclusions were reached by Hoagland (4), who indicated that the yield is largely a condition of the supply of nutrients during the first few weeks of growth, and is but little affected by the later uptake. On the other hand, the absence of phosphate from the nutrient solution, just at the time tillering was due to begin, inhibited the formation of ears without reducing tiller production until the phosphate had been withheld for a rather longer initial period. This finding as to the vital necessity of phosphate in the early stages of development appears to run directly contrary to the conclusion of Pember and McLean (6), who found that barley could utilize a deficient supply of phosphate equally well whether it was supplied early, in the middle period, or late in the life of the plants. The explanation of this discrepancy may lie in the fact that well water was used throughout the American experiments, providing a certain amount of phosphate in every case over and above that used experimentally. It may be suggested that the actual requirement of the plant for phosphorus in the earliest days is exceedingly small, and that even a minimal amount supplied at this time ensures the proper laying down of the ears, which can then develop later, when more plentiful supplies of phosphate become available. In the total initial absence of phosphate for short periods the energy of the plant appears to be directed towards abundant tiller production at the expense of the ears, a tendency that would seem to be counteracted by the presence of small quantities of phosphate at this time. A further point to be considered is that Pember and McLean removed all tillers as they appeared, and only allowed the main shoot to develop. Consequently, any small supply of phosphate was concentrated in its sphere of action, and may have induced ear formation in cases in which it would have been insufficient if tillers had been allowed to develop. This is further exemplified by the Rothamsted plants, grown entirely without phosphate, which produced no tillers but developed one feeble ear plant, presumably at the expense of the phosphate contained in the seed.

The presence or absence of phosphorus from the nutrient solution at the time tillering actually begins has a definite effect upon the later development. Plants that were grown with and without phosphate for alternate fortnights throughout their life-history showed differences in development according to whether phosphate were present or absent during the second

fortnightly period, in which tillers began to appear. With phosphate *present* at this time, the plants were distinctly ahead throughout growth, the ears emerged some days earlier, and the weight of the grain and the total dry weight were greater than when phosphate was *absent* at the critical moment. In the latter case, the ears did not all emerge completely even when ripe, giving the plants a characteristic appearance of poor growth (Pl. III, Fig. 3). In both sets the dry weights of the grain, shoot, and the whole plant were considerably lower than when phosphate was supplied for the first six weeks and then withheld—i.e. over the whole period of eighteen weeks better growth was obtained with one-third the total possible supply of phosphate provided consecutively during the first six weeks than from one-half the supply provided intermittently throughout growth. This, again, may emphasize the importance of the early weeks, as in the alternating series less phosphate was available during these weeks than in the case of the others, although their total supply was greater.

Dry Weight.	+P first 2 weeks then −P, +P alternately.	−P first 2 weeks then +P, −P alternately.
	grm.	grm.
Shoot	13·58	15·88
Leaves	8·03	7·00
Root	3·18	2·60
Grain	1·11	3·18
Sterile flowers	0·50	0·60
Total	26·40	29·26

Compare +P for 6 weeks, then −P.
Dry weight 34·62 grm.

There would appear to be a certain limit of possible growth which is reached if phosphate is available for a certain consecutive period at the beginning of growth, but which is not attained if it is entirely withheld even for the first fortnight only, nor if an intermittent supply of phosphate is continued throughout growth.

Gericke (3), working with wheat, obtained the maximum dry weight when the plants were grown in complete nutrient solutions for four weeks and were then transferred to solutions devoid of phosphorus. Decrease occurred in the ultimate dry weight as the initial period with phosphate was lengthened, the plants receiving phosphate throughout being amongst those with the lowest dry weight. Barley, under Rothamsted conditions of growth, did not show a sharp fall to correspond with this, though the plants transferred after eight weeks with phosphate (corresponding in stage of development to the wheat at four weeks' old) were somewhat heavier than those transferred later. The difference, however, is relatively small when the error of experiment is taken into account. In the following table the two plants are compared, the first transfer being made at corresponding stages of development.

Wheat. With P. for	Barley. With P. for	Wheat. (Gericke.)	Barley. (Brenchley.)
Weeks.	Weeks.	grm.	grm.
4	8	51·2	37·2
6	10	41·7	31·8
8	12	40·7	32·6
10	14	31·4	32·3
Throughout	Throughout	32·0	35·0

The more closely the work of various investigators is studied, the stronger grows the impression that the requirement of cereals for phosphate at different growth periods is by no means fixed or definite, but is influenced by various factors, such as environmental conditions (season, spacing of plants, time of sowing, &c.), inter-action of other nutrients, and the degree of completeness of phosphate starvation induced at different periods of growth. Nevertheless, whatever the condition, the indications are that the most critical period for phosphate nutrition is during the early stages of growth, and that later provision of phosphate tends to affect the composition of the crop without having a corresponding effect upon yield.

Summary.

1. Experiments have been made in water cultures to test the effect of depriving barley plants of phosphorus after varying initial periods during which it had been supplied, and, contrariwise, of supplying phosphorus after initial periods of deprivation.

2. The provision of phosphate for the first six weeks or longer permitted normal growth to be made, as was shown by the number of tillers, ears, and grains produced, the average number of grains per ear, and the dry weights. With shorter initial periods of phosphate supply growth was seriously depressed in all these respects.

3. If phosphate was withheld for the first four weeks, tiller production was not affected, but no ears were produced. With longer periods of initial deprivation growth was steadily depressed in all respects, and the type of growth gradually changed from a bushy succulent character to a thin, lanky, untillered plant bearing the travesty of an ear.

4. The amount of phosphate absorbed by the plant increased steadily in more or less direct proportion to the length of time phosphate was given at the beginning of growth, but sufficient was taken up in the first six weeks to enable the plant to make its maximum dry weight. The percentage of phosphate in dry matter rapidly increased from this time onwards.

5. The absence of phosphate supply up to the first six weeks of growth caused a extremely rapid drop in the amount of phosphate ultimately taken up by the plant, after which a more gradual decrease occurred with lengthening periods of phosphate deprivation.

6. The significance of the above results is discussed in relation to the

work of other investigators, and the probable importance of the presence or absence of phosphorus at the time tillering begins is indicated by reference to further experiments in which phosphate was supplied and withheld for alternate fortnights during growth.

My sincere thanks are due to Miss Katherine Warington, M.Sc., Miss Lizzie Kingham (both of Rothamsted), and Miss F. L. Stephens, B.Sc. (of Manchester) for assistance in the tedious work of marking and counting tillers; to Miss Signe Heinze, of Stockholm, for aid in translation; and to the Chemical Department of Rothamsted for the series of phosphate determinations, without which the paper would have lost much of its point.

Appendix A

Data of Barley Plants grown with or without Phosphate for the first part of their Life history.

(Averages of five replicates.)

Weeks with or without Phosphorus.	No. of Ears.	Dry Weight.						Percentage P_2O_5 in Dry.			Actual P_2O_5.			
		Grain.	Sterile Flowers.	Shoot.	Leaves.	Root.	Total.	Grain + Sterile flowers	Shoot + Leaves.	Root.	Grain. + Sterile flowers	Shoot + Leaves.	Root.	Total.
		grm.	grm.	grm.	grm.	grm.	grm.				grm.	grm.	grm.	grm.
+P 18 (All time)	15·6	6·233	0·365	18·122	6·460	3·840	35·020	1·660	0·98	3·560	0·1095	0·2409	0·1367	0·4871
+P 16	17·0	5·997	0·347	17·914	7·438	3·466	35·162	1·830	0·99	3·330	0·1161	0·2510	0·1154	0·4825
+P 14	14·2	4·968	0·277	16·650	7·258	3·138	32·291	1·860	1·02	2·890	0·0976	0·2439	0·0907	0·4322
+P 12	14·6	5·623	0·291	17·546	6·126	3·032	32·618	1·750	0·77	1·700	0·1035	0·1823	0·0515	0·3373
+P 10	15 4	4·517	0·396	16·656	7·256	2·976	31·801	1·740	0·68	0·732	0·0855	0·1626	0·0218	0·2699
+P 8	16·8	6·977	0·433	19·558	7·126	3·068	37·162	1·460	0·39	0·494	0·1082	0·1041	0·0152	0·2275
+P 6	14·6	6·948	0·178	17·694	6·672	3·132	34·624	0·655	0·13	0·303	0·0467	0·0317	0·0095	0·0879
+P 4	7·4	1·270	0·213	6·896	3·360	2·344	14·083	0·456	0·23	0·247	0·0068	0·0236	0·0058	0·0362
+P 2	1·6	0·038	0·039	1·019	0·770	0·523	2·389	—	0·16	0·197	—	0·0029	0·0010	0·0039
+P 0 = −P 18	1·0	—	0·004	0·148	0·121	0·083	0·356	—	0·20	0·137	—	0·0005	0·0001	0·0006
−P 16	1·0	0·006	0·011	0·225	0·075	0·138	0·455	—	1·74	0·940	—	0·0054	0·0013	0·0067
−P 14	1·0	0·024	0·017	0·164	0·131	0·104	0·440	—	2·60	1·320	—	0·0087	0·0014	0·0101
−P 12	0·8	0·013	0·016	0·317	0·381	0·193	0·920	—	2·18	2·360	—	0·0159	0·0045	0·0204
−P 10	—	—	—	0·422	0·464	0·182	1·068	—	2·64	1·580	—	0·0234	0·0029	0·0263
−P 8	—	—	—	1·54		0·176	1·716	—	2·39	2·730	—	0·0368	0·0048	0·0416
−P 6	—	—	—	0·886	1·808	0·313	3·007	—	2·15	4·050	—	0·0579	0·0127	0·0706
−P 4	—	—	—	3·910	7·462	1·066	12·438	—	2·37	3·430	—	0·2694	0·0366	0·3060
−P 2	15·6	3·975	0·496	16·210	6·998	3·282	30·961	1·960	1·12	4·240	0·0877	0·2599	0·1392	0·4868
−P 0	15·6	6·233	0·365	18·122	6·460	3·840	35·020	1·660	0·98	3·560	0·1095	0·2409	0·1367	0·4871

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EXPLANATION OF PLATE III.

Illustrating Dr. W. E. Brenchley's paper on Phosphate Requirements of Barley.

Fig. 1. Barley plants which received phosphate at first, but were deprived of it after varying periods of growth.

Upper line of figures $+P$ indicates initial time with P.
Lower ,, ,, ,, $-P$,, later time without P.

Fig. 2. Barley plants grown without phosphate at first, but which were supplied with it after varying periods of growth.

Upper line of figures $-P$ indicates initial time without P.
Lower ,, ,, ,, $+P$,, later time with P.

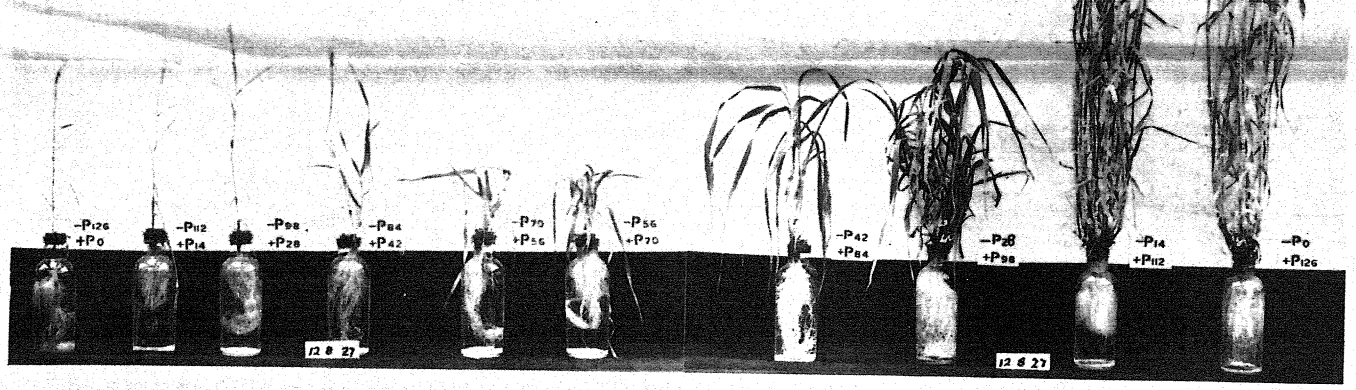
Fig. 3. Barley grown with and without phosphate for alternate fortnights throughout growth.

Left-hand pair—without P for first fortnight.
Right-hand ,, with P for first fortnight.

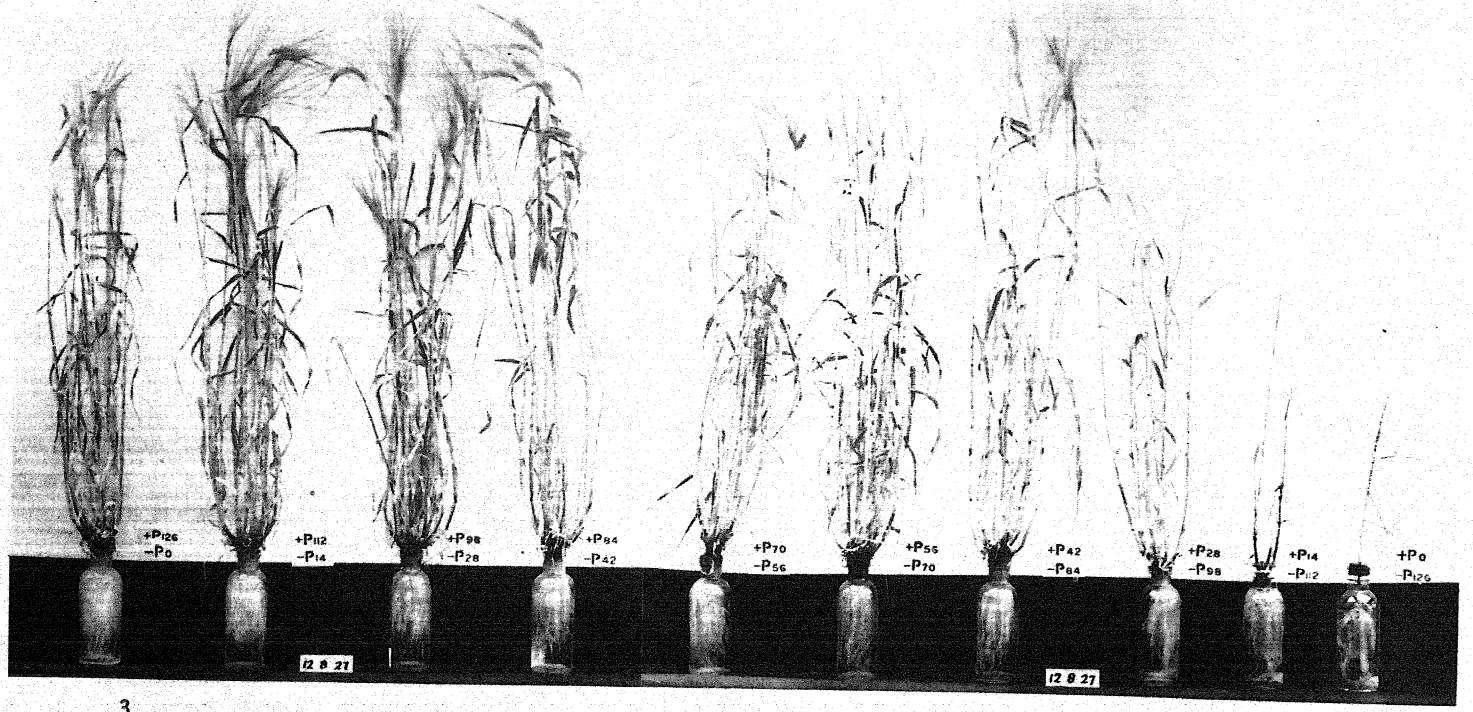
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BRENCHLEY — PHOSPHATE REQUIREMENT OF BARLEY.

Huth, London.

Gametogenesis and Fertilization in Achlya racemosa.

BY

MARGERY C. CARLSON.

With Plate IV.

INTRODUCTION.

ACHLYA RACEMOSA was named and described by Hildebrand in 1867. Previous to this time, De Bary (4) and Pringsheim (16, 17, 18) had made extensive studies of the morphology of the genus *Achlya* and of related genera. Pringsheim (19, 20) maintained that fertilization occurs, at least, in some species, but De Bary (6) concluded that the Saprolegniaceae as a group are parthenogenetic and that antheridia, even though present, are functionless. Humphrey (12) and Hartog (7, 8) described a pairing and fusion of the nuclei in the oogonia which, they thought, replace fertilization. Trow (22, 23, 24, 25), in spite of much opposition, held to the fact of functional sexuality in *Saprolegnia dioica*, De B., *S. mixta*, De B., *Achlya americana*, Humphrey, *A. polyandra*, Hild., and *A. De Baryana*, Humphrey. In 1904 Mottier (14) said, 'The sexuality of the Saprolegniaceae is one of the oldest questions in botany still in dispute'. Since this time, however, fertilization has been demonstrated in *S. monoica* (Prings.), De B. by Claussen (1), in *Aphanomyces laevis*, De B. by Kasanowsky (13), and in *A. polyandra*, De B. by Mücke (15).

MATERIAL AND METHODS.

The material from which the species used in this investigation was isolated was collected at the state fish hatchery at Hayward, Wisconsin. It was reported as occurring on debris and on the sides of the concrete tanks, but not on the fish. It was isolated by Mr. J. A. Lounsbury, using the single zoöspore method, and identified by him as *A. racemosa*, Hildebrand.

The stock cultures were grown on pea-extract agar. Cultures for study were made by placing a small cube of agar from the stock culture in sterile, distilled or tap water in a sterile watch-glass or petri dish. Ant

[Annals of Botany, Vol. XLIII. No. CLXIX. January, 1929.]

larvae, dried pieces of beef, or sterilized pieces of corn kernels were then dropped into the dishes. Usually an attempt was made to bring the substratum in contact with the agar cube, in order to permit the mycelium in the agar to infect the new substratum directly.

When the culture had reached the required age or stage of development, as determined by microscopic examination, it was carefully lifted, together with the substratum, and dropped into the fixing solution. Flemming's weak solution, with 2 per cent. urea, diluted to one part of solution in three, four, or five parts of distilled water, chrom-acetic solution, full strength and diluted, Merkel's solution, as recommended by Smith (21), and a mixture of 6·5 c.c. formalin, 2·5 c.c. acetic acid, and 100 c.c. 50 per cent. alcohol, full strength, and in several dilutions were used. None of the fixatives used gave uniformly good results. The best preparations obtained were fixed in the formalin-acetic-alcohol solution, diluted to one part in five parts of water.

Flemming's triple stain, often omitting orange G, and Heidenhain's iron-alum-haematoxylin were used, but the best results were obtained with the former.

Sections were cut 5, 10, 12, or 15 μ in thickness.

Observations.

Short branches arise laterally, in racemose order, near the ends of hyphae in cultures three days old. The tips of the branches enlarge to form spherical swellings, which become completely filled with cytoplasm (Pl. IV, Fig. 1). Many nuclei pass from the main hyphae into these enlarged tips. Each nucleus is spherical, surrounded by a membrane, and contains a centrally placed nucleolus and peripheral chromatic masses. Threads extend from the peripheral masses to the nucleolus across a clear region (Pl. IV, Figs. 2–5).

A difference of opinion has existed among investigators regarding the nature of the body which I have called the nucleolus. Hartog (8) interpreted it as a chromatin body, and Trow (22, 24) thought it a combination of nucleolus and chromosome. Davis (2), Claussen (1), and Mücke (15) agree in calling it a nucleolus.

A vacuole appears in the developing oogonium and becomes continuous with the vacuole of the hypha (Pl. IV, Fig. 6). Finally, a cell-division occurs, separating the oogonium, which consists of the terminal swollen portion and a short neck, from the main portion of the hypha. A wall is then formed between the oogonium and hypha (Pl. IV, Fig. 11). The central vacuole of the oogonium becomes spherical. The oogonial nuclei enlarge. The chromatic material of each nucleus becomes dispersed throughout the nuclear cavity, the threads become thicker, and the peripheral masses smaller (Pl. IV, Fig. 9).

I did not find a small deeply-staining body, just within the nuclear membrane, such as was described by Claussen (1) as a 'Zentralkörper' and by Mücke (15) as a centrosome.

A large number of the nuclei in the young oogonium now disappear. Hartog (8) and Humphrey (12) accounted for this decrease in the number of nuclei by the continued fusion of the nuclei in pairs. Trow (22), Davis (2), Claussen (1), and Mücke (15) thought that most of the nuclei degenerate. I have found no evidence of fusions, but have seen many nuclei which do not show the typical organization. These may be degenerating.

The antheridial hyphae develop as slender branches from the oogonial hypha (Pl. IV, Fig. 6). Usually two antheridial branches appear opposite each other, arising soon after the enlargement of the tip of the oogonial hypha begins. *Achlya racemosa* is peculiar in that an antheridial branch may originate either below or above the place where the basal wall of the oogonium will be, or it may arise from the spherical part of the oogonium.

The tip of an antheridial branch enlarges and attaches itself by its end to the developing oogonium. This attachment occurs before the oogonium is separated by cell-division from the hypha on which it is borne (Pl. IV, Fig. 6). Many nuclei migrate into the swollen tip of the antheridial branch; the tip is then cut off from the slender filament by a wall (Pl. IV, Fig. 23, above). The nuclei in the antheridium resemble those in the oogonium in size and structure. The number of nuclei usually decreases.

The persisting nuclei in the oogonium and antheridium enlarge and prepare to divide. The chromatic material of each nucleus becomes aggregated into definite masses scattered throughout the nucleus, and connected by delicate threads (Pl. IV, Fig. 10). The nucleolus decreases in size and eventually disappears. The masses of chromatic material become smaller and the threads which connect them become thicker, forming the spireme (Pl. IV, Figs. 10–13). The spireme becomes more and more uniform in thickness, as can be noted from a comparison of Figs. 10, 12, and 13. Earlier investigators did not describe a definite spireme.

The spireme breaks transversely into chromosomes. A spindle is formed within the nuclear membrane. The fibres (or groups of fibres) are thick and conspicuous. The chromosomes are moved to the equatorial region (Pl. IV, Figs. 14–17). They are distinct, rod-shaped bodies which might with careful study at high magnification be counted with accuracy. Davis (2) finds four chromosomes in *Saprolegnia mixta*, Trow (24) apparently eight in the first mitosis and four in the second in *Achlya De Baryana*, Claussen (1) ten to fourteen in *S. monoica*, and Mücke (15) probably more than eight in *A. polyandra*. My figures suggest that the number is small, probably four or six.

The chromosomes divide and the daughter chromosomes are moved to the poles of the spindle (Pl. IV, Figs. 18–20). The nuclear membrane is

not evident in anaphases. Finally, the daughter nuclei are organized, but very soon most of them disappear.

The question as to whether or not a reduction in the number of chromosomes occurs in the division, or divisions, of nuclei in the oogonium is still unanswered. Trow (24) is the only investigator who has found two divisions, and he maintains that the second is a reduction division. Since most of the daughter nuclei disappear soon after the division, its significance is still further complicated.

Changes in the shape of the central vacuole of the oogonium indicate the beginnings of egg-formation (Pl. IV, Fig. 21). The cytoplasm becomes concentrated into more or less hemispherical masses, each one of which is destined to become an egg. These masses are connected at their bases by a thin layer of cytoplasm. Claussen (1) says that the vacuole, by its furrowing through the cytoplasmic layer, is the active agent in the cutting out of the egg origins. Davis (2) attributes the 'balling' of the egg origins to 'dynamic activities' in the cytoplasm at the centre of each origin. He describes a coenocentrum, which appears when the egg origins begin to form, as the morphological expression of these dynamic activities.

A single nucleus lies at the centre of each egg origin, all other nuclei having disorganized. The chromatin is largely confined to the periphery of the nucleus. No nucleolus is evident. A dark-staining body, with radiations at the peak of the pear-shaped nucleus, was often found in an egg origin. This body is somewhat similar to that described by Davis as a coenocentrum, and by Claussen and Mücke as a centrosome and aster (Pl. IV, Fig. 22, *a*). Fig. 22, *b*, indicates that the body and radiating lines are cytoplasmic. It was not observed in all cases (Pl. IV, Fig. 21).

The vacuolar extensions finally reach the oogonial wall, thereby breaking the cytoplasmic layer which connected the egg origins. The one to eight eggs thus formed now mass together in the centre of the oogonium, pressing against each other and becoming flattened on adjacent sides. Later they contract, become spherical, and come to lie scattered throughout the oogonium (Pl. IV, Fig. 23). Each egg is surrounded by a very thin wall. It contains dense cytoplasm, enclosing several large vacuoles, granules of various sizes, and a single centrally placed nucleus. I found no indication of centrosomes or asters attached to the nuclei in young eggs.

The fertilization tube begins as a bulge from the antheridium, which pushes through a narrow opening in the oogonial wall (Pl. IV, Fig. 23). The protuberance elongates until it comes into contact with an egg (Pl. IV, Figs. 23–7), or branches and grows towards two or more eggs. The tube may be of equal diameter throughout its length, as shown in Figs. 23 and 27, or it may increase in diameter near the end, as shown in Fig. 24.

One to several nuclei move from the antheridium into the tube. The one nearest the tip, destined to be the male gamete nucleus, enlarges

(Pl. IV, Fig. 24). The nuclei remaining in the antheridium probably degenerate (Pl. IV, Fig. 24).

An open communication between a fertilization tube and an egg is seen in Figs. 23, 25, 26, and 27. The female gamete nucleus usually lies at the centre of the egg. It is sometimes spherical, sometimes oval, and often irregular in shape. It contains no nucleolus. The chromatin, apparently much diminished in quantity, is dispersed as a layer just within the nuclear membrane or aggregated in small masses connected by fine threads (Pl. IV, Fig. 23).

A male gamete nucleus passes into an egg, migrates directly to the female gamete nucleus and fuses with it. Fig. 23 shows a male gamete nucleus just leaving the fertilization tube, and Figs. 25 and 26 show the two gamete nuclei lying in contact, but not yet fused. An accident to the slide during observation caused the fertilization tube to be pulled out of the egg in the oogonium shown in Fig. 25. A part of the cytoplasm of the oosphere was pulled out with the tube, showing that the connexion between them must have been close. Fig. 27 shows a zygote in which the gamete nuclei have fused. After the fusion of the two gamete nuclei a gradually thickening wall is secreted about the zygote.

I have made no study of the changes which accompany the ripening and germination of the zygote, although such studies should prove very interesting in their relation to the reduction division. If it is true that no reduction in chromosome number occurs during nuclear division in the oogonium and antheridium, it is possible that such a reduction may occur during some of the nuclear divisions following fertilization.

Summary.

1. *Achlya racemosa*, Hild., is monoecious, producing oogonia and antheridia from the same branch.

2. The development of the sexual organs is the same as that described for related forms by previous investigators.

3. One mitotic division of nuclei was found in the oogonium and antheridium. There is no indication that it is a heterotypic division.

4. A fertilization tube from the antheridium enters an egg and discharges one nucleus which fuses with the egg nucleus. This adds a sixth species to the Saprolegniaceae in which fertilization has been demonstrated.

My thanks are extended to Dr. E. M. Gilbert and to Dr. C. E. Allen for suggestions and criticisms during the progress of this work.

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EXPLANATION OF PLATE IV

Illustrating Dr. M. C. Carlson's paper on Gametogenesis and Fertilization in *Achlya racemosa*.

All drawings were made with the help of the Abbe camera lucida. All figures of nuclei were drawn with a Zeiss 2 mm. apochromatic oil-immersion objective and a no. 18 compensating ocular (magnification × 3,800). All other figures were drawn with the same objective and a no. 6 compensating ocular (magnification × 1,200).

Fig. 1. Young oogonial hypha.

Figs. 2–5. Resting nuclei from oogonial hyphae. Figs. 2 and 3 from the oogonial hypha shown in Fig. 1. Figs. 4 and 5 from a younger oogonial hypha.

Fig. 6. Developing oogonium, showing central vacuole. Young antheridial branch.

Fig. 7. Nucleus from the antheridium shown in Fig. 6.

Fig. 8. Nucleus from the developing oogonium shown in Fig. 4.

Fig. 9. Nucleus of young oogonium.

Figs. 10, 12. Oogonial nuclei in the early spireme stage.

Fig. 11. Oogonium, showing nuclei in the late spireme stage. × 950.

Fig. 13. One nucleus of Fig. 11, enlarged.

Fig. 14. Oogonium, showing nuclei in metaphase; one nucleus in anaphase.

Figs. 15–17. Nuclei in metaphase. Fig. 17 from the oogonium shown in Fig. 14.

Figs. 18–20. Nuclei in anaphase.

Fig. 21. Egg origins.

Fig. 22, *a* and *b*. Nucleus, with centrosome and aster (?) from an egg origin. Fig. 22*a* × 1,200; Fig. 22*b* × 3,800.

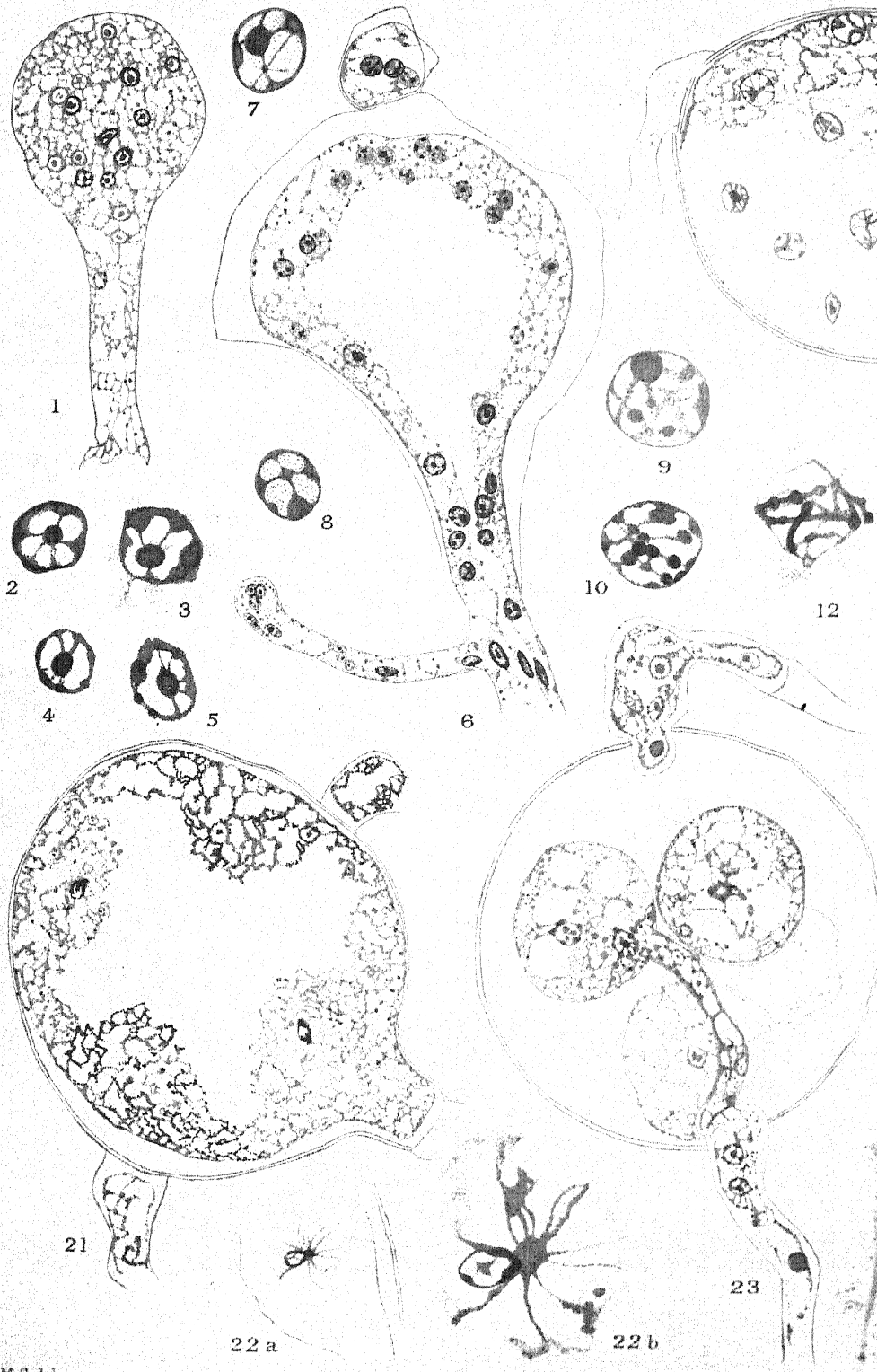
Fig. 23. Eggs. One fertilization tube which has just pushed through the oogonial wall. A second fertilization tube in open communication with the egg. Female gamete nucleus near the centre of each egg. Male gamete nucleus just discharged from the fertilization tube.

Fig. 24. Antheridium and fertilization tube. Male gamete nucleus at the tip of the fertilization tube.

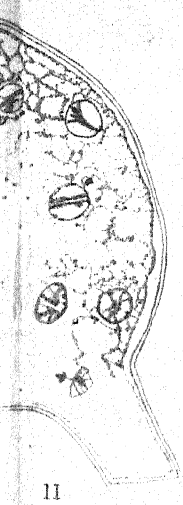
Fig. 25. Gamete nuclei in contact. Fertilization tube accidentally pulled out of egg.

Fig. 26. Gamete nuclei in contact.

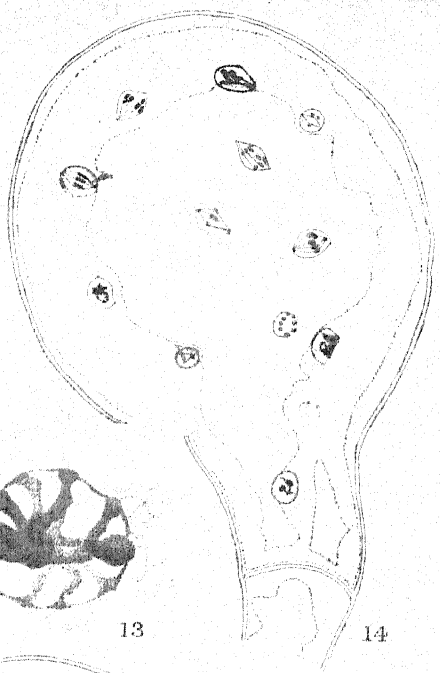
Fig. 27. Fusion nucleus. Young zygote.



CARLSON—ACHLYA.



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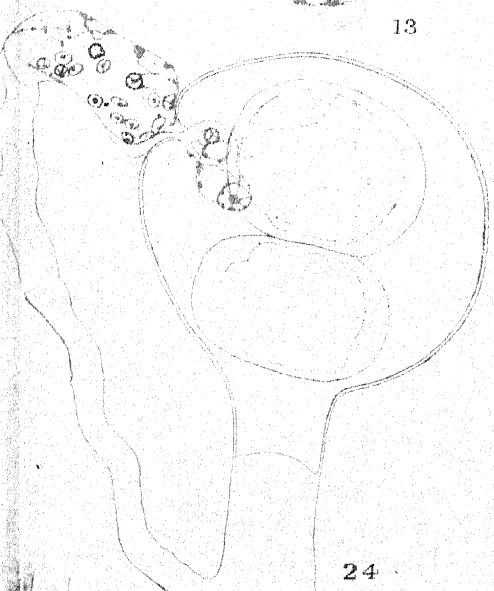
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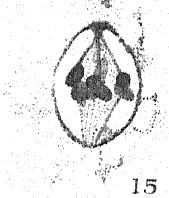
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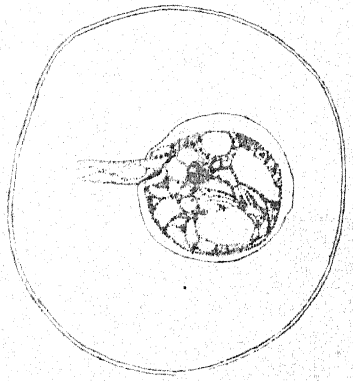
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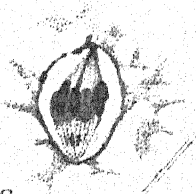
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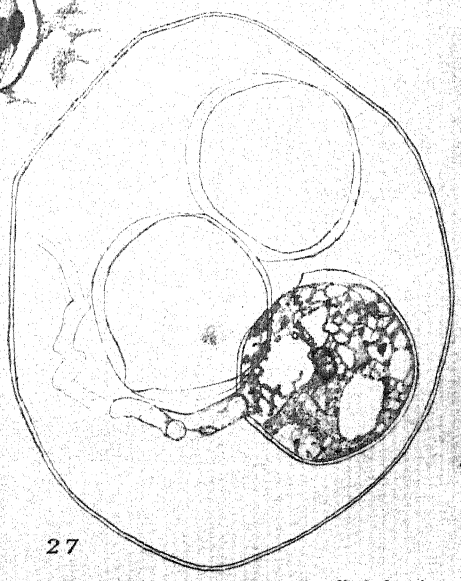
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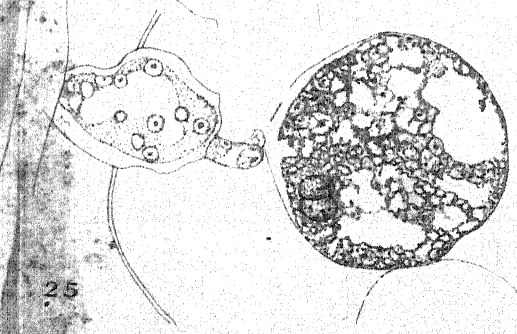
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Huth, London.

Physiological Studies in Plant Nutrition.

I. The Effect of Manurial Deficiency on the Respiration and Assimilation Rate in Barley.

BY

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AND

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(*From the Department of Plant Physiology, Imperial College of Science and Technology, London.*)

With sixteen Figures in the Text.

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INTRODUCTION.

IN view of the great importance of the problem of plant nutrition, and the amount of attention which the relations of plant yield to manurial applications have received in the past, it is surprising how little work has been published dealing with the effects of various manurial constituents on

[Annals of Botany, Vol. XLIII. No. CLXIX, January, 1929.]

such fundamental processes as respiration and photosynthesis. Concerning the assimilation rate, the only work of this kind known to the authors is that of Briggs, who found that deficiency of any of the essential elements (Fe, Mg, or K) led to a lowering of the assimilation rate, a result which he interpreted in terms of change in 'reactive chloroplast surface'. It will appear in the sequel that the evidence presented by Briggs is not conclusive, and, indeed, for phosphate and potash starvation does not appear to be in accordance with the experimental data he brings forward. Examination of these data will be undertaken later, when they will be dealt with in the light of the evidence presented in the present paper.

The effect of manurial deficiency on respiration rate does not seem to have received attention from physiologists in the past, though the effect of solutions of various substances on the respiration of mature plant organs has been studied. Thus Lyon (8) determined the effect of placing Elodea in solutions of phosphates, and found an increase in respiration rate. Since death occurred within twenty-four hours, it is probable that the toxic action of phosphates due to secondary effects was being studied rather than the part which phosphate ions play in the general metabolic processes.

The aim of the present series of experiments was twofold: (1) to determine the general course of respiratory and assimilatory activities of leaves in succession throughout the life-cycle of plants adequately supplied with the necessary manurial elements in properly balanced proportions; and (2) to determine the modification of the course of respiration and assimilation induced by deficiency in each of the three elements, nitrogen, phosphorus, and potassium.

The investigation here reported finds its place in a wide scheme of work on the physiological effects of manurial constituents, which has been in progress for some years. By the analysis of growth data information has been gained of the net assimilation rate of plants grown in various culture media, and it was felt that the conclusions thus reached should be tested by further work in the laboratory, dealing specifically with the problems of assimilation and respiration under controlled conditions. The general conclusions from growth data which will appear in subsequent papers of this series have been corroborated by the laboratory work, a fact which lends much support to the methods of growth analysis employed and indicates the validity of results obtained by such methods.

Experimental Procedure.

The plants used in this investigation were grown in the open air, in sand culture, in glazed pots each holding 30 lb. of sand, as previously described (18). The variety Plumage Archer was used throughout, and

four manurial series were employed, the following amounts of pure salts per pot being added to each set respectively:

	Na_2HPO_4.	$NaNO_3$.	K_2SO_4.
Fully manured	2·52	9·1	1·85
Phosphate deficient	0·504	9·1	1·85
Nitrate deficient	2·52	1·82	1·85
Potash deficient	2·52	9·1	0·20

In addition to all series were added 0·37 grm. $CaCl_2$ per pot.
1·25 grm. $MgSO_4.7H_2O$.

The seeds were sown on 9th May 1927, and germinated on 18th May. On successive days each week the youngest leaf on the main stem which had reached full development was selected, removed from the plants, and taken to the adjacent laboratory in a stoppered glass tube lined with moist filter paper.

The first determinations were made on 8th–11th June, one on each day in the following order: potash deficient, fully manured, phosphate, nitrate deficient. Each week these four determinations were repeated in the order stated, until the last leaf on the main shoot was dying. The last experiment on the nitrate deficient series was carried out on 18th August, those on fully manured and phosphate deficient the following week, while the potash series yielded one further determination. The first experiment in each case utilized the second and third leaves on the main shoot; the next five weeks provided determinations on the successive leaves from the third to the seventh week; by the eighth week the eighth leaf was already too mature for use, and the ninth leaf was therefore used, as also for the following experiment in the ninth week, while all subsequent determinations were made on the tenth leaf. The last determinations, therefore, follow the senescent change in activity of the last leaf, rather than the ageing of the plants as a whole.

Method of determining Assimilation and Respiration Rates.

The Katharometer. The apparatus used for determining the amount of CO_2 given out or taken up by the leaf was the katharometer. This instrument, as adapted to plant physiological measurements, has been described by Waller (15), and a general account is therefore unnecessary here. In several particulars, however, our experience of the instrument differs from that of Waller. Two types of katharometer have been tried, the compensated type (as used by Waller), and the older asymmetrical form, but the former appears to possess no marked advantages over the latter; it certainly is not compensated, though in construction it may be symmetrical. In order to obtain true compensation, the first requisite is that the electrical parts of the instrument, and particularly the platinum spirals, be properly balanced; this was not the case in either of the instruments used by us.

Evidence of this was provided by altering the strength of the heating current passing through the instrument, the strength advised by the makers being 120 milliamps. Assume that with this current flowing, conditions are such that a deflection is produced in the galvanometer. If the instrument is properly balanced electrically, and the heating current is gradually reduced to zero, the galvanometric deflection should also simultaneously come to zero; but even in the compensated instrument this did not hold: the current in the galvanometer circuit fell to zero a long time before that in the main circuit, and then reversed, producing a large deflection in the opposite direction; finally it reversed again, coming to zero.

This effect of the asymmetry is unimportant, as the heating current is kept constant; but it has other, and more troublesome, results. Thus, if both arms of the katharometer are connected to the same chamber, containing air, and the pressure inside this chamber is altered, then the deflection changes; this should not be the case in a compensated instrument. Also, very slight changes of temperature affect the deflection markedly. The result of this sensitivity to so many factors was apparent when the katharometer was set up as described by Waller, though with both arms connected to the same air chamber; the zero of the instrument, instead of remaining constant, showed a slight but continual drift—usually in one direction through the day, reversing during the night. Although this was undoubtedly largely the result of temperature change, it was found impossible to correlate it with this with sufficient accuracy to allow of a correction from a temperature record; on some days when the temperature was more uniform than usual the drift would be greater than the average, and vice versa. This drift proved very troublesome, and it was only by enclosing the katharometer in a double-walled asbestos-lined metal box packed with cotton wool, and the leaf chamber in a large volume of water, and working with the whole apparatus in an underground constant temperature room, that it was reduced to reasonable dimensions. Even with these precautions, the zero drift in extreme cases might amount to as much as 4 per cent. of the respiration rate. One obvious method of reducing the relative value of the drift was to enclose the leaves in as small a chamber as possible, but this proved to have strict practical limits, as will be seen shortly.

Another factor which appears to have been more troublesome to us than to Waller is the lag period of the instrument. This will obviously depend on the length of the diffusion path from the leaf chamber to the spiral, and also on the cross-sectional area of that path. The diffusion path used was as short as practicable and apparently shorter than that used by Waller, if it is possible to judge from his published diagram (Fig. 2). The platinum spirals of the katharometer normally have communication with the outside air only by three very fine tubes drilled through the solid brass case; in the compensated instrument used these tubes were very much enlarged, in

order to reduce as far as possible the resistance to diffusion, and consequently the lag of the instrument. In spite of this, the lag period was very long, considerably over an hour being required, after a switch over from light to darkness, before the maximum rate of movement in the opposite direction (that of respiration) was attained. On reversing these conditions, and changing from a state of respiration alone to one of assimilation, the lag was not so great, the maximum speed being attained usually after about forty minutes. This means that the amount of CO_2 in the chamber at the beginning of an assimilation period must be sufficiently great to allow assimilation to proceed at its maximum rate for at least this length of time. This introduces a limit to the smallness of the leaf chamber compared with the leaf surface used, since if the chamber is very small, the concentration of CO_2 at the beginning of illumination would be toxic. In practice, a compromise between these two considerations was made, and a chamber of such a size used that the CO_2 concentration need never rise above 4–5 per cent.

Whether Waller's instrument actually showed a shorter lag period, or whether the rates of respiration and assimilation as measured by him are short of the actual rates, is not quite clear. A study of his galvanometric record of alternate light and dark periods (Fig. 4) shows that he took his readings for respiration rates within half-an-hour from turning off the light, and for assimilation rates after about ten minutes of illumination. If a 'straight-edge' is placed against the three respiration tracings, it is found that the first is irregular (presumably because the instrument had not been left for a sufficient length of time to settle down), while the two last show a continually increasing rate throughout the whole of the dark period; the correct rate of CO_2 production is presumably not at the point where it has been measured, but would only have been recorded had the respiration been allowed to continue for a longer time than was actually the case. The three assimilation tracings are too nearly vertical to attempt to draw any conclusion from them, but it would seem highly probable that nowhere on the record has the actual rate been reached, CO_2 becoming the 'limiting' factor before the lag period has ended; in other words, these curves are sigmoid in form, having no straight portion between the two opposite curvatures.

Waller states that, when the atmospheric pressure round one spiral varied while that round the other was constant, a change of '1 per cent. produced a momentary galvanometric deflection which almost immediately subsided, while even with a change of 4 per cent. the deflections were not of much account'. Preliminary observations of the effect of pressure change in one arm of our instrument showed that a change of 1 per cent. produced a deflection (which did *not* subside until the pressure was released) of about 9 mm., i.e. approximately equivalent to that produced by three parts of CO_2 in 10,000 of air. This appears at first sight considerable, but under

the conditions of working (as Waller has also shown for his apparatus), even assuming as low respiratory and assimilatory ratios as 0·5, the change of pressure caused thereby would only affect the results by 1 per cent. In all probability the error due to such a cause is actually much less than this.

Calibration. The instrument was calibrated for CO_2 by connecting one arm (the one to which the leaf chamber is usually attached) to a large vessel containing air to which has been added a known quantity of CO_2. A change of concentration of one part of CO_2 in 10,000 of air produced a deflection of 3·05 mm. Respiration and assimilation rates calculated according to this calibration are too high, since the effect of the changing concentration of O_2 has not been taken into account. Assuming that the respiratory and assimilatory coefficients are unity, the results will be 10 per cent. too high, since, according to Daynes, the effect of O_2 upon thermal conductivity is one-tenth that of CO_2, and is in the opposite direction. This correction has been applied to all the results.

Leaf Chamber. The leaf chamber finally used consisted of a rectangular block of aluminium with two depressions, 10 × 4 × 1 cm. deep; over these was placed a piece of glass, the joint being made secure by means of a vaselined india-rubber washer to which the glass was clamped. The depressions thus formed two chambers, the leaves to be experimented upon being placed in one, mounted on a piece of glass which loosely fitted into it; the other was furnished with a similar piece of glass. The chambers were connected one to each arm of the katharometer by means of two tubes let through the walls of the aluminium block, and finally the whole block was sunk in a large water-bath, this latter having a polished glass front.

When this chamber was first used, in running blank experiments, it was found that the galvanometer moved as fast as in a normal respiration experiment. The aluminium was evidently giving off some gas, in all probability hydrogen, to which the katharometer is known to be extremely sensitive. This was largely overcome by painting over the metal surface with shellac, but it was not until a coat of white enamel had been applied that all movement ceased.

The volume of the leaf chamber as actually used in the experiments, together with the air space in the katharometer surrounding the platinum spiral, was determined by removing a known volume of air, and finding the change of pressure caused thereby. The volume thus obtained was 35·7 c.c.

The light used in the assimilation experiments was provided by three Mazda gas-filled bulbs (150 watts, 110 v.), immersed in running water, and placed a few inches from the leaf chamber; the light from these was concentrated by means of a large condenser lens. Although precautions were taken to absorb as much as possible of the heat from these lamps, it was found impossible altogether to prevent them affecting the katharometer.

However, the greatest effect was produced shortly after switching on the light, the drift caused thereby gradually subsiding, until after half an hour it was well under 1 per cent. of a normal assimilation rate. In determining the light intensity at the compensation point (at which assimilation just balances respiration), one lamp only was used, without the condenser lens, and its distance from the leaves was varied until the deflection of the galvanometer remained constant.

In these experiments, portions only of the leaves could be used; strips about $3\frac{1}{2}$ in. long were cut from the central region, each experiment being performed on at least two leaves from different plants. The cut ends were protected by a film of vaseline, by which means the tendency to lose water in the chamber was largely eliminated. Under these conditions also, no increase in the rate of respiration due to wounding was observed; on the contrary, during the first few hours after cutting the leaves, the rate of respiration usually showed a slight but continual falling off. In one instance some leaves were left in darkness in the chamber for several days, and the respiration rate determined every afternoon. The first day gave the highest rate (13·1), and a falling one, which by the second day had reached 10·5. The following three days showed a gradual rise to 12·3, and the next two a fall to 10·0, after which mould appeared on the leaves. The general form of the respiration curve seems to be that found for leaves under conditions of starvation.

Experimental Procedure. The experiments were carried out as follows: the leaves were cut and the apparatus set up at 11 a.m.; it was then left to settle down, five-minute readings of the respiration rate being started at 2.30 p.m. These readings were continued for two and a half hours, after which the light intensity at the compensation point was determined by the method described above. The light was again extinguished, and the CO_2 of respiration allowed to accumulate until late in the evening. When this had reached a concentration of 4–5 per cent., the assimilation rate at the high light intensity was determined; the rate of movement of the spot of light over the galvanometer scale proved so rapid that it was usually found more accurate to observe the time taken to move equal distances than to take galvanometer readings at equal intervals of time. At the conclusion of the experiment the fresh weight, the leaf area (by means of a planimeter), and the dry weight of the leaves were determined.

The temperature at which the experiments were performed depended on that of the underground room in which the apparatus was housed. Throughout the series the maximum variation was about 4° C., and this was more or less progressive through the summer; the respiration and assimilation rates were corrected to an approximate mean value of 17° C. by using a coefficient of 2·0 for a rise of 10° C.

Before going on to describe the experimental results it may be well to

give a record of actual values obtained in a typical experiment, in order to give some indication of the accuracy with which estimates of assimilation and respiration rates may be made with the katharometer when sufficient precautions are taken to eliminate sources of error.

Experiment 2. Fully manured series, Thursday, 16th June 1927. Leaves removed from plants 11.30 a.m., in chamber 12 noon.

Respiration, 5-minute readings.			*Assimilation.*		
Time.	Reading on Scale.	Rate (Scale Divisions).	Time (Seconds) for a Deflection of 20 mm.		
p.m.					
3.5	167·1	—	53	49	46
3.10	178·8	11·7	52	48	46
3.15	190·0	11·2	53	49	46
3.20	201·1	11·1	52	48	46
3.25	212·7	11·6	51	47	46
3.35	223·8	11·1	50	47	—
3.40	235·0	11·2	50	46	—
3.45	246·0	11·0	51	48	—
3.50	257·1	11·1	50	46	—
3.55	268·3	11·2	49	47	—
4.0	279·5	11·2	49	46	—

The figures show the steady respiration values obtained, while the gradual rise in assimilation rate to a steady maximum value is also apparent. It would seem that accurate measures of assimilation rate could be made in as short a space of time as a minute, and of respiration in five minutes.

Experimental Results.

Various Data. Before dealing with the respiration and assimilation rates of these leaves, it may be interesting to note the course taken by various other characteristics of the leaves in the four series throughout the season of which the values are given in Table I. The data are given for each of the four types of manuring used for successive leaves on the main shoot throughout the vegetative cycle. These values are presented graphically in Figs. 1, 2, and 4. In Fig. 1 is shown the ratio of dry weight to leaf area. Generally speaking, this is higher in the nitrate and phosphate deficient series than in the fully manured, while it is considerably lower in the case of potash deficiency. The general form of curve is quite similar in all four types, the level being determined by the manuring, and the 'period' of the oscillations about the mean level by the length of the life-history of the plants—this latter itself, of course, being dependent on the manuring. The highest values obtained for the ratio were in the nitrogen deficient series, and the lowest in the potash deficient. The curves in all four cases show a rise during the first week, then a fall to a minimum (the drop occupying one, two, three, and six weeks respectively in the four

TABLE I.

$\frac{\text{Weight of Water}}{\text{Leaf Area}}$ = mg. water per dm. Leaf Surface.

$\frac{\text{Dry Weight}}{\text{Leaf Area.}}$ = weight in mg. per dm.^{2} Leaf Surface.

Leaf No.	Fully manured.			Potash deficient.			Nitrogen deficient.			Phosphate deficient.		
	Wt. of Water. / Leaf Area.	Wt. of Water. / Dry Wt.	Dry Wt. / Leaf Area.	Wt. of Water. / Leaf Area.	Wt. of Water. / Dry Wt.	Dry Wt. / Leaf Area.	Wt. of Water. / Leaf Area.	Wt. of Water. / Dry Wt.	Dry Wt. / Leaf Area.	Wt. of Water. / Leaf Area.	Wt. of Water. / Dry Wt.	Dry Wt. / Leaf Area.
2 and 3	205·3	5·31	38·6	239·5	7·57	31·6	195·7	5·79	33·8	191·6	4·72	40·8
3	219·2	5·20	42·2	227·8	6·24	37·2	212·3	5·83	37·0	229·7	5·44	43·3
4	209·6	6·36	33·4	232·8	6·65	35·0	206·2	5·88	35·1	252·3	5·91	42·7
5	234·7	6·84	34·4	256·9	8·73	29·4	225·8	5·35	42·2	251·9	6·80	37·0
6	194·7	6·25	31·2	199·9	7·26	27·5	163·9	3·41	48·1	219·7	5·57	39·5
7	141·4	3·59	39·4	177·0	6·89	25·7	139·3	3·02	46·3	148·6	3·19	46·5
9	146·7	3·50	42·0	154·1	5·76	26·7	146·0	3·40	42·9	142·1	3·31	42·9
9	133·2	3·60	37·0	141·4	5·51	25·6	124·8	2·77	45·0	132·8	3·11	42·7
10	100·9	2·55	39·7	105·5	3·29	32·1	93·2	2·27	41·2	92·6	2·22	41·7
10	99·6	2·58	38·6	114·8	3·38	34·0	96·7	2·72	35·5	92·9	2·31	40·2
10	95·4	2·57	37·0	104·7	3·48	30·1	89·4	2·59	34·5	86·6	2·06	42·0
10	69·9	2·02	34·6	121·3	3·74	32·4	—	—	—	86·8	2·08	41·7
—	—	—	—	118·8	3·51	33·9	—	—	—	—	—	—

types) followed by a rise to a maximum two weeks later, and a subsequent decline. In the way these curves are plotted, the four maxima lie nearly on a straight line; in other words, the value of this ratio at the maximum is an inverse function of the time taken to reach this stage. With the exception of the phosphate deficient series (and the leaf used in the fourth determination of this series was somewhat abnormal) [1] the minima in these curves also lie on a straight line.

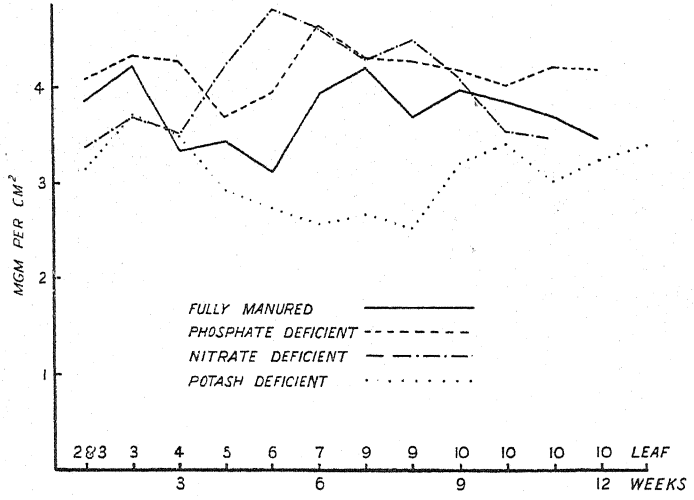


FIG. 1. Graph showing weight in mgm. per square cm. of leaf surface of the leaves of the four series.

The water content showed the usual responses obtained with these types of manuring (Fig. 2). The plants starved of potash had a very much higher, while the phosphate and nitrate deficient had a rather lower, average water content than the fully manured series. Throughout the life-history, in all four types, the general form of curve is fairly constant; there is a rise to a maximum at the fourth week, or fifth leaf (earlier in the nitrate deficient), and a subsequent decline, this latter (with the exception of the potash deficient series) occurring in more or less well-marked steps, chiefly between the sixth and seventh (fifth and sixth in the nitrate deficient) and between the eighth and ninth leaves.

At first sight, there is very little connexion between the water-content curves and the curves for the ratio of dry weight to leaf area (Figs. 1 and 2), but a close inspection shows that the inter-relationship in the water-content curves of any two series is the inverse of the inter-relationship in the dry weight to leaf area curves of the same two series. This is particularly well marked in the case of the nitrate deficient and the fully manured series.

[1] The basal portion of this leaf only was used as the tip was already yellow and dry.

Not only is the nitrate deficient curve generally below that of the fully manured in one case, and above it in the other, but the two pairs cross over twice at identical points, and a close approach in one pair always coincides

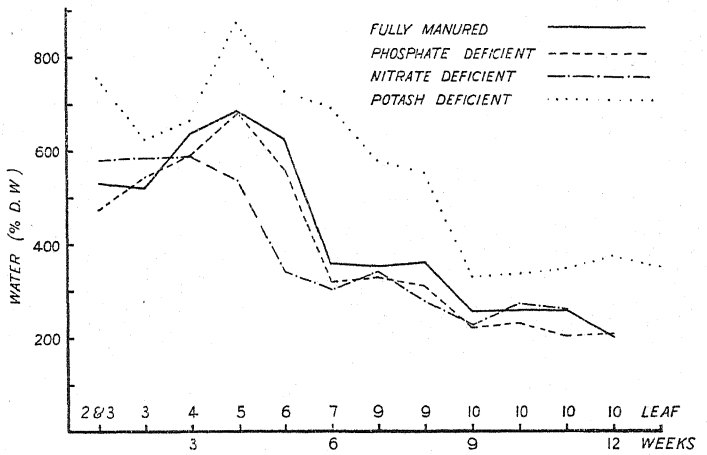


FIG. 2. Graph showing water content as percentage of dry weight in leaves of the four series.

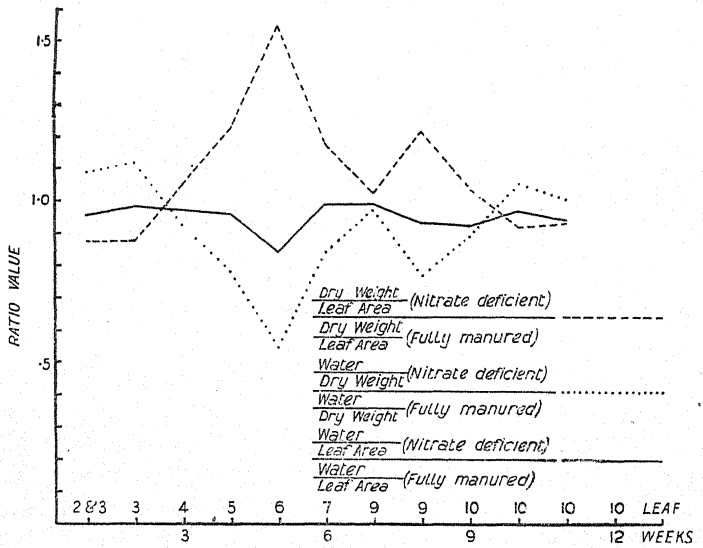


FIG. 3. Graph showing various relationships of water content and dry weight in the fully manured and nitrate deficient series. For description see text.

with a close approach in the other. This relationship in these two manurial types is better shown in Fig. 3, where the ratio of the water content in one series to that in the other is drawn, together with the ratio of dry-weight-per-unit-leaf-area in one to that in the other. The third curve is obtained by multiplying together corresponding points of the other two, and its

approach to horizontality is a measure of the inverse proportion existing between them. Now the product of these other two ratios is merely the ratio between the weight-of-water-per-unit-leaf-area in the nitrogen deficient and that in the fully manured series, which is thus shown to be nearly constant. In the same way, a very high correlation can be shown to exist

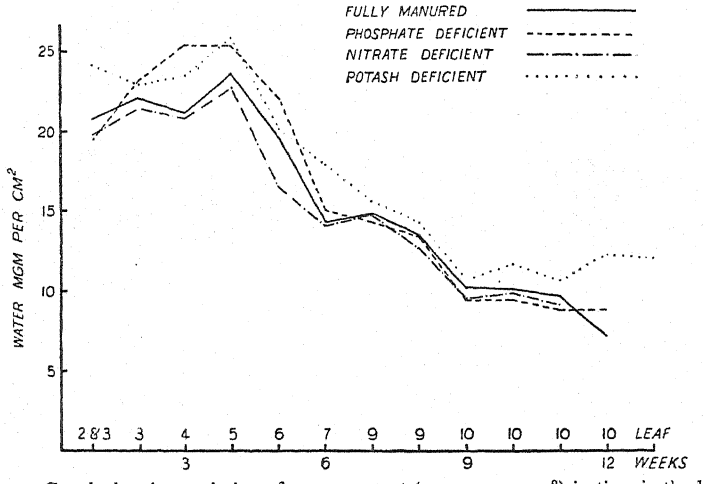


FIG. 4. Graph showing variation of water content (as mg. per cm.^{2}) in time in the leaves of the four series.

between the weight-of-water-per-unit-leaf-area in the potash deficient and the fully manured series throughout the season, and a lesser correlation between the phosphate deficient and the fully manured.

Thus, in all four series (with the exception of a few early points in the phosphate deficiency curve) the amounts of water per unit area vary together much more uniformly than do the water contents as measured in the usual way, on a dry weight basis. This is shown in Fig. 4; the general course of these curves is very similar to that of the water-content curves, but the four are much closer together, both in general level and also—at least in the case of the nitrogen deficiency—as to the time of the maximum and the times at which the greatest amounts of water are lost. The potash deficient leaves still have their water content (on an area basis) very slightly higher than the fully manured, and the nitrogen deficient very slightly lower. The only points which are thrown further out are a few of the early ones in the phosphate deficient series; in this case, the water content, instead of being uniformly rather below that of the fully manured, as on a dry weight basis, is at first higher, crossing over and becoming lower at about the eighth leaf.

These considerations appear to indicate that in the case of leaves, area provides a better basis for the calculation of water content than does dry weight.

Respiration Rate. The rates of respiration corrected for temperature are presented in Table II, calculated on both the dry weight and leaf area bases. Graphs of these values on a dry weight basis are shown in Fig. 5. When plotted on the basis of leaf area, the general course is very similar, though with a steeper drop in the early portion; the relative positions of the four are also nearly the same, though those of the phosphate and nitrate deficient series are generally raised very slightly compared with that of the fully manured, and that of the potash deficient series is lowered, particularly near the middle part of the curve. Precisely the same differences are brought out as in the curves shown, though to a slightly less degree.

TABLE II.

Rates of Respiration.

Respiration on Dry Weight Basis gives mg. CO_2 per hour per grm. Dry Weight
„ „ Leaf Area „ „ „ „ „ sq. dec. Leaf Surface per hour.

Leaf No.	Fully Manured.		Potash Deficient.		Nitrogen Deficient.		Phosphate Deficient.	
	Dry Wt. Basis.	Leaf Area Basis.	Dry Wt. Basis.	Leaf Area Basis.	Dry Wt. Basis.	Leaf Area Basis.	Dry Wt. Basis.	Leaf Area Basis.
2 and 3	4·49	1·734	5·93	1·881	4·15	1·399	4·95	2·020
3	4·24	1·822	5·33	1·978	3·56	1·319	3·80	1·647
4	2·81	0·937	4·77	1·671	3·26	1·144	4·01	1·713
5	2·77	0·950	4·23	1·243	2·00	0·851	2·64	0·975
6	2·78	0·868	4·20	1·152	1·64	0·789	2·91	1·155
7	2·65	1·042	3·81	0·987	1·62	0·743	1·81	0·840
9	2·38	0·996	4·16	1·109	2·07	0·886	2·28	0·979
9	2·65	0·981	4·56	1·166	1·98	0·891	2·78	1·193
10	2·86	1·133	4·71	1·506	2·31	0·952	3·28	1·364
10	2·26	0·870	4·38	1·492	2·20	0·780	2·72	1·093
10	2·68	0·990	3·80	1·143	2·40	0·829	2·56	1·077
10	2·52	0·873	4·45	1·442	—	—	2·57	1·068
10	—	—	4·47	1·518	—	—	—	—

The general shape of the curve in the fully manured series indicates a rapid fall in the rate of respiration during the first two weeks of the observations, subsequent determinations showing very little further change. In the three deficient series, the initial drop was more protracted, respiratory activity reaching a minimum at the seventh leaf, and subsequently showing some measure of recovery. It should be remembered that all these determinations (with the exception of the last few in each series) were made on the youngest fully-formed leaf, the points giving, therefore, the approximate values of the respiration rates of the successive leaves at corresponding early ages.

The four curves give conclusive evidence that the respiration rate of the young leaves varies with manuring, and that different manurial constituents affect the rate in different ways. On a dry weight basis, phosphate deficiency has no marked effect on the rate, this curve and that of the fully

manured crossing over eight times in twelve determinations. The mean percentage value, compared with the fully manured series, is 103·6 per cent., rising to 116·8 per cent. on a leaf area basis. Nitrate deficiency produces a marked drop in the respiration rate, the only determination in this series

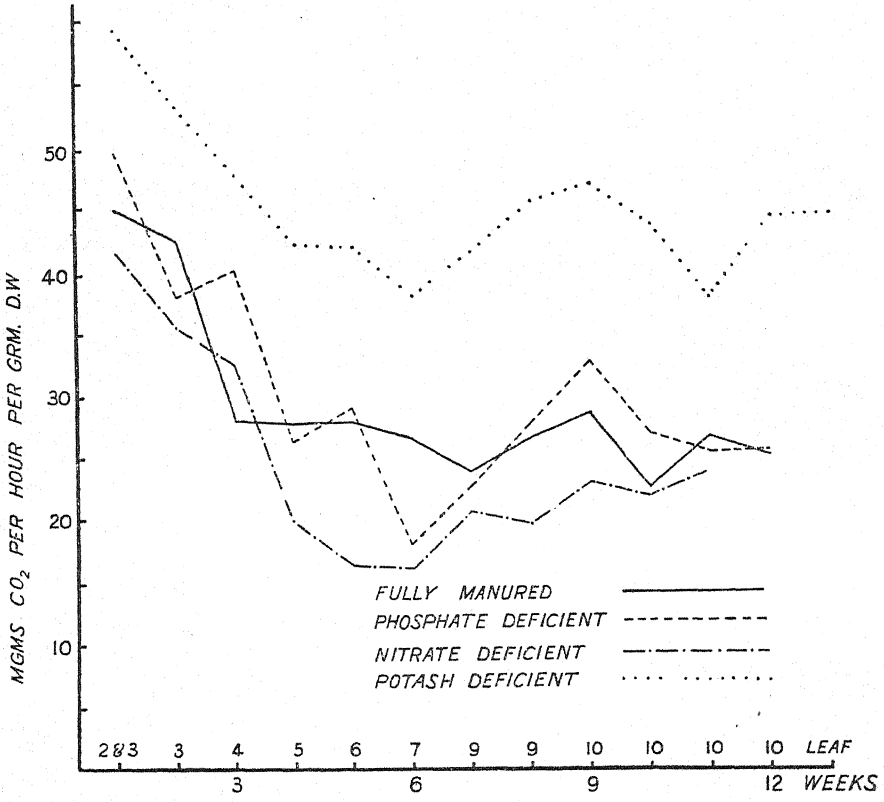


FIG. 5. Graph showing rates of respiration on a dry weight basis of leaves of the four series.

exceeding the corresponding one in the fully manured being the third. The mean percentage value throughout the season compared with the latter was 83 per cent., and 87·7 per cent on a leaf area basis—on this basis, also, the third week was the only one giving results in the reverse order. Deficiency of potash, on the other hand, produces a very marked rise in the rate of respiration, the mean value, on a dry weight basis, being 158·3 per cent. of that in the fully manured series; the rate is well in excess even on a leaf area basis, in spite of the much lower value of the ratio of dry weight to leaf area in this series, i. e. 130·6 per cent., the increase being approximately half that on the dry weight basis. It is interesting to note that, considering the three deficient series, the rates of respiration as determined each week were invariably in the same order—in an ascending series, nitrate, phosphate, and

potash deficient. This is true also for the nitrate deficient, fully manured, and potash deficient, with the single exception at the third determination already noted.

The results which have been presented qualitatively above may also be dealt with quantitatively. There are clearly two factors at work, namely, (1) the effect of age of plant inducing a fall in respiration in all the series, (2) the effect of manurial deficiency modifying the effect of the first factor. In order to disentangle the effects of these two factors, and further to obtain an estimate of error of the determinations, Fisher's Analysis of Variance (4) has been used. To obtain a symmetrical table the first eleven values of each series alone have been employed. In essence the method makes use of the fact that the total variance of the figures about their mean value may be divided into two parts: that due to known causes, in this case age and manuring, and a residue due to unknown causes (the experimental errors). The variance attributable to these causes may then be tested against each other to determine whether the effects obtained differ significantly from the variance to be expected from the unaccountable variation (experimental error). For this purpose, Fisher's Z test (4) has been used. The results obtained are given below.

Analysis of Variance for Respiration Rate.

(Dry Weight Basis.)

Mean Value of 11 Respiration Rates.

Fully manured	2·961	Mean = 3·259.
Nitrogen deficient	2·472	
Phosphate deficient	3·067	
Potash deficient	4·535	

	Degrees of Freedom.	Sum of Squares.	Mean Square.	Log Mean Square.	Standard Deviation.
Age	10	21·2733	2·1273	0·75482	—
Treatment	3	26·0952	8·6984	2·1638	—
Remainder	30	2·6584	0·08861	−2·42351	0·2977 (9 %)
Total	43	50·0269	—	—	—

Z (Age remainder)	1·5892	1 % point = 0·555
Z (Manuring remainder)	2·2933	1 % point = 0·753

The result of the analysis shows very clearly that the variance due to manuring, as well as to age, are significantly different from variance due to experimental error,[1] and the odds are far greater than 100 : 1 against the result obtained arising by chance. It may therefore safely be concluded that the fall of respiration rate in successive leaves as the plant ages is real, and further, that the respiration rates of leaves from plants differently manured vary with the treatment. The estimate of experimental error from the 30 degrees of freedom of the Remainder makes possible a direct

[1] The 1 % point in the Z distribution is the value of Z which has for the given number of degrees of freedom a significance of 100 : 1. Both values of Z obtained are of very much higher significance.

test of the differences between the means of the separate manurial treatments. The value of the standard error is seen to be 12·0 per cent. of the mean respiration rate, indicating that two determinations of respiration on similar leaves from two plants identically manured may differ by 24 per cent. without such differences being significant. This result indicates the variability of the material used, and shows that the minor fluctuations on the curves are due only to variability of the material. Caution is thus required in drawing deductions from experiments if only very few determinations are made, since the differences found may be solely due to unavoidable variability of the plant material used. The value of the standard error appropriate for differences between means of groups of eleven will be $= 0{\cdot}0608$.

Comparing the manurial treatments in turn with the fully manured the results are as follows:

	Differences of Means.
Fully manured – Nitrogen deficient	$+0{\cdot}489 \pm 0{\cdot}127$
Fully manured – Phosphate deficient	$-0{\cdot}106 \pm 0{\cdot}127$
Fully manured – Potash deficient	$-1{\cdot}574 \pm 0{\cdot}127$

Nitrogen and potash deficient series thus differ from the fully manured by more than twice the standard error and are thus significantly different in their respiration rates; nitrogen deficient leaves having a lower, and potash starved a higher respiration rate than the fully manured. The value for experimental error obtained from the Analysis of Variance will be a maximal value, since all the secondary interactions of age and manuring, represented by the varying positions of minima on the curves, and themselves a cause of variance about the mean, are included in the experimental error. The Z test is the most reliable means of estimating the relative effects of the causes of variance, and the direct test on differences of the means are merely confirmatory.

Assimilation Rate. Briggs claims to have shown that with manurial deficiencies, assimilation rate is subnormal both in the photochemical and dark phases. Bearing this possibility in mind, the experiments here described were carried out at two light intensities (Table III), one low enough to ensure that light was limiting. The high light intensity remained constant throughout, while for estimation of assimilation at low light intensity the compensation point was determined, as previously described. The distance of the single lamp from the leaf chamber was recorded at each determination of the compensation point. To convert these measurements of distance into relative light intensities, and also for purposes of comparison with the high light intensity, the actual intensities for the varying distances and the two illuminations were determined in two ways. The first method consisted in enclosing a Hilger linear thermopile in the leaf chamber in the position normally occupied by the leaves, and under conditions exactly as used in

the assimilation experiments. The radiation intensity was measured by means of a galvanometer in series with the thermopile. This method gave comparative results only in so far as the proportion of light radiation and heat radiation transmitted by the water screens remained unchanged. To test this matter further the light intensity was determined directly with a lumeter, placing the standard white surface inside the leaf chamber as before. The curves obtained by the two methods were identical, and hence the energy values were known both in terms of calories per unit area per unit of time, and metre candles.

TABLE III.

Assimilation Rates on Leaf Area Basis: mg. CO_2 per. sq. dec. per hour.

Leaf No.	Fully Manured.		Potash Deficient.		Nitrogen Deficient.		Phosphate Deficient.	
	Low Light.	High Light.	Low Light.	High Light.	Low Light.	High Light.	Low Light.	High Light.
2 and 3	1·368	17·61	1·496	18·39	1·491	18·22	1·530	16·64
3	1·465	20·26	1·241	15·01	1·345	17·75	1·658	20·03
4	1·131	13·87	1·140	13·64	1·448	13·73	1·579	15·54
5	1·219	16·01	0·529	4·49	1·288	11·52	1·540	16·24
6	1·258	12·00	0·544	4·00	1·573	11·93	1·471	14·29
7	1·523	12·05	1·572	12·37	1·443	10·02	1·491	9·63
9	1·515	9·96	1·112	8·60	1·492	12·59	1·453	13·21
9	1·502	12·77	1·500	11·59	1·385	4·80	1·501	14·66
10	1·355	10·56	1·349	9·48	1·193	7·01	1·424	14·96
10	1·377	8·99	1·570	12·09	1·125	9·19	1·477	12·61
10	1·373	12·19	1·334	11·21	0·914	8·51	1·260	10·53
10	0·784	6·08	1·165	10·21	—	—	1·014	8·53
10	—	—	0·614	5·27	—	—	—	—

The figures obtained in the two calibrations are given below:

(*a*) Linear thermopile.

Distance from lamp	6 in.	8 in.	10 in.	12 in.	14 in.	16 in.	18 in.
Light value (galvanometer deflection)	96·0	66·1	45·9	34·8	26·9	22·1	18·5

(*b*) Lumeter.

Distance from lamp	6 in.	8 in.	10 in.	12 in.	14 in.	16 in.	18 in.
Light value (foot candles)	83	55	40	29	22	18	15

If the logarithms of the sets of values of the two variables are plotted the curves obtained are very nearly straight lines, and moreover of almost identical slope in the two cases. Thus the tangents of the slopes of the lines are 1·56 and 1·62 respectively. By interpolation in these curves the light intensity at any distance from the lamps was read off, and by conversion of the galvanometer deflection into metre candles (since the intensity of light was too high to read direct on the lumeter), the high light intensity used was found to be 5,000 metre candles approximately, while the light intensity at the average of the various compensation points was found to be 300 metre candles.

In order to make direct comparisons of the assimilation rates at high and low light intensity, the values of assimilation rate at the compensation point were all reduced to the average light intensity at the compensation point. The value of the assimilation rate at this point is known to be equal to the respiration rate, which has been previously determined. A slight uncertainty is introduced by the small variations in temperature in the different experiments, but it is clear that under these conditions light must be the 'limiting factor'. Further, whatever temperature was actually recorded for a particular experiment, the corresponding respiration rate gives at once without correction the value of assimilation rate strictly comparable with those of other experiments, irrespective of the temperatures prevailing at the time they were performed. The assimilation rates at the compensation points were therefore reduced to a common light intensity by a process of simple proportion, the values of respiration uncorrected for temperature being used in each case. The full justification for this procedure will be given later, but at present it will be seen that the only assumption made is that at the compensation point light is the 'limiting factor'; and hence that in this region assimilation rate is proportional to light intensity.

TABLE IV.

	Respiration.	Assimilation.	
		Low Light Intensity.	High Light Intensity.
Fully manured	—	Unaffected by age of plant	Falling with age of plant
Nitrogen deficient	*Subnormal*	Normal; unaffected by age of plant	Subnormal; falling with age of plant
Phosphate deficient	Normal	Slightly supernormal; *falling with age*	Slightly supernormal; falling with age
Potash deficient	*Supernormal*	*Subnormal*	*Subnormal*

Table III presents data of assimilation rates at high and low intensities of light calculated on the basis of mg. CO_2 per sq. decimetre per hour. The ratio of leaf area to dry weight given in Table I make possible, if required, a conversion of the data to the basis of dry weight.

The rates of assimilation (real) under conditions of high light intensity for the four types of manuring are given graphically in Figs. 6–8, calculated on the leaf area basis.

(a) *Assimilation Rate at High Light Intensity.* The general form of the curve in the fully manured series is somewhat similar to the respiration rate curve, showing a continual fall in the early part of the season, and becoming almost horizontal after the sixth or seventh determination; the decline in the assimilation rate is therefore more gradual than that in the respiration rate.

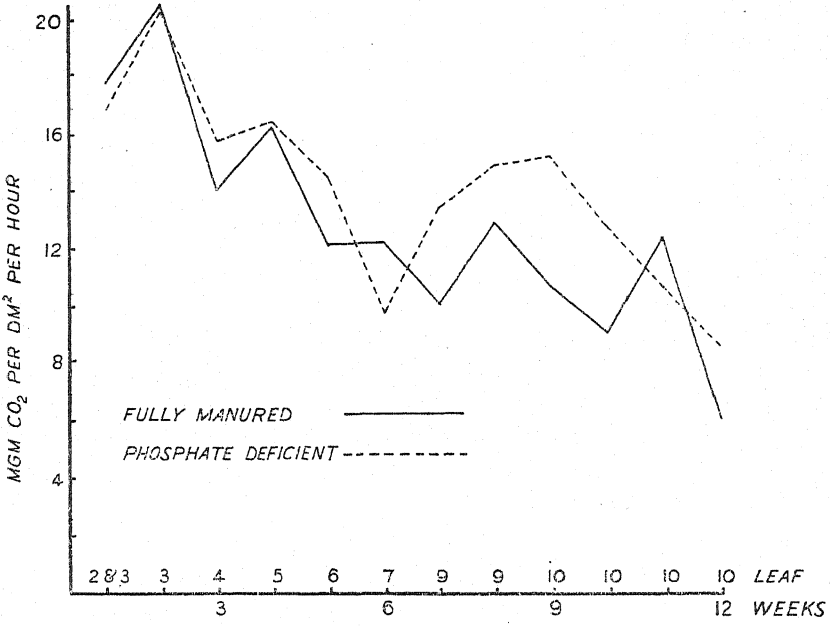


FIG. 6. Graph showing rates of assimilation of leaves of phosphate deficient and fully manured series under high light intensity.

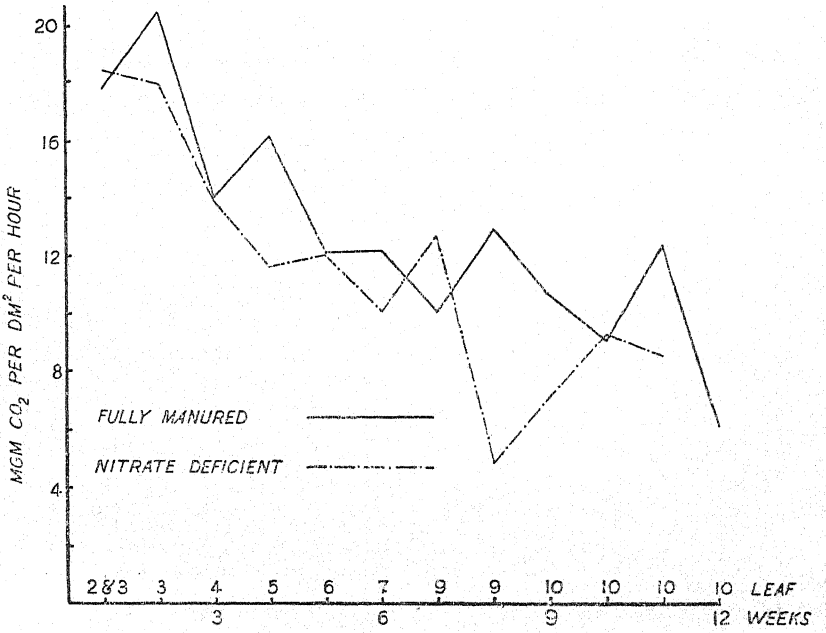


FIG. 7. Graph showing rates of assimilation of leaves of nitrate deficient and fully manured series under high light intensity.

Manurial deficiency apparently affects the rate of assimilation in these young leaves to a much less degree than it affects the respiration rate; but again, the curve of each deficient type shows a definite minimum point, with a subsequent recovery, of which there is no very clear evidence in the fully manured series. Phosphate deficiency certainly does not decrease the assimilation rate, and there is some indication that it causes a slight increase; thus the average percentage rate in this series, compared with the fully manured, on a leaf area basis, is 111·1 per cent.; on a dry weight basis, however, the mean value is 100·4 per cent. (in these and later values the final determinations are ignored, as they are often obviously abnormal, and were made on leaves which were rapidly dying). As can be seen from Fig. 6, there is very little difference between the two rates until after the minimum at the seventh leaf, when the recovery in the phosphate deficient series pulls up its average figure. On a dry weight basis this curve is uniformly slightly below that of the fully manured until the minimum is reached, subsequent points being generally higher—in fact, the inverse relationship to that obtaining in the corresponding curves of the amounts of water per unit leaf area. It is interesting to note that this minimum occurs at precisely the same point as that in the respiration rate curve for this series; both processes subsequently rise to a maximum at the ninth determination, finally falling off together. The general agreements between respiration and assimilation holding throughout the life-histories in the fully manured and phosphate deficient series (and a similar agreement can be traced, though not so well, in the other two deficient series) are interesting in the bearing they may have on the conclusions of such workers as Plester (11), concerning green and yellow varieties, Henrici (6), dealing with alpine and lowland plants, and Boysen-Jensen (1), who find that in plants of the same species, those with a high photosynthetic rate generally have also a high respiration rate, and vice versa. Turning again to the minima in these two rates at the sixth determination of the phosphate series, it may be pointed out that they occur at precisely the same point as the maximum in the ratio of dry weight to leaf area; this, however, is probably unimportant, since the same relationship cannot be traced in the other series. It is more likely to be significant that these minima occur about the time of the cessation of tillering and the beginning of increase in length of the stems.

In the case of the nitrate deficient series (Fig. 7), a slight decrease in the average rate of assimilation is indicated; the mean percentage value of the rate of the fully manured series is 88·2 per cent. on a leaf area basis, and 84 per cent. on a dry weight basis. Here a minimum is not reached until late in the life-history—the eighth determination, while only eleven were possible before the last leaf had withered.

Potash deficiency has a very decided effect on the general shape of the

curve (Fig. 8), but not, apparently, on its mean height. The curve shows a very marked drop in the assimilation rate at the fourth and fifth determinations, with a subsequent recovery to the value of the fully manured series. Apart from this drop, the general course is similar to that of the latter. On a leaf area basis the mean value is 84·5 per cent. of that of the fully

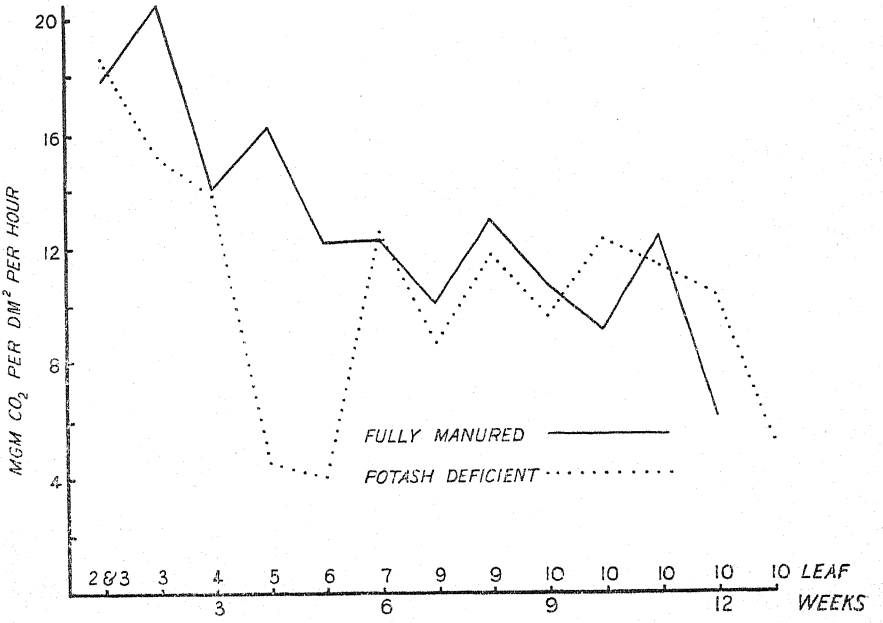


FIG. 8. Graph showing rates of assimilation (mgm. CO_2 per dm.^{2} per hour) of leaves of potash-deficient and fully manured series under high light intensity.

manured, or, neglecting the abnormally low fourth and fifth determinations, 96·5 per cent.; thus, apart from this temporary collapse, potash starvation does not depress the assimilation rate to any significant extent. On the other hand, on a dry weight basis, the recovery after this collapse takes the assimilation rate well above that in the fully manured series; even including the two low values the mean is 106·6 per cent. of the latter, and, neglecting them, is as high as 122·6 per cent.

(b) *Assimilation at Low Light Value.* To bring out in the clearest possible manner the relations of the assimilation rates at the two light intensities, they are presented together for each type of manuring in Figs. 9–12. The scale for the low assimilation rates has been increased ten times, a consideration which must be borne in mind in estimating the significance of the fluctuations of the low light intensity curves.

In general for the fully manured series, and the phosphate and nitrogen deficient sets, the contrast between the two curves is very marked. There is no clear fall in assimilation rate at low light intensity in the fully

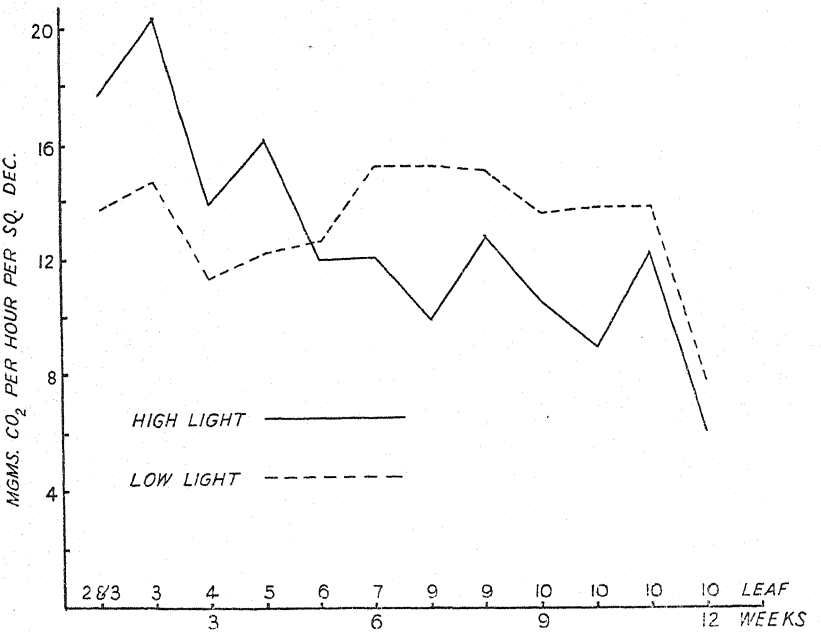


FIG. 9. Graph showing assimilation rate of leaves of fully manured series at high and low intensities of light.

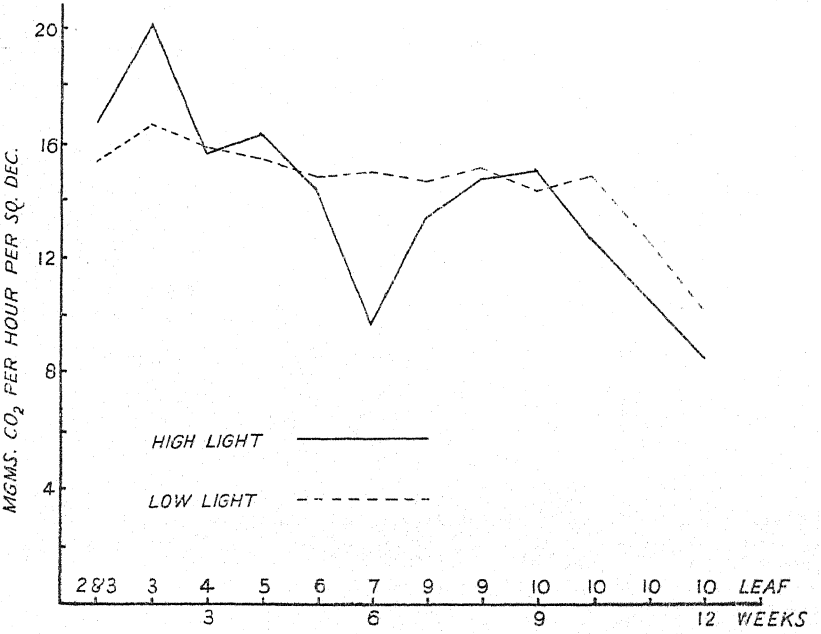


FIG. 10. Graph showing assimilation rate of leaves of phosphate deficient series at high and low intensities of light.

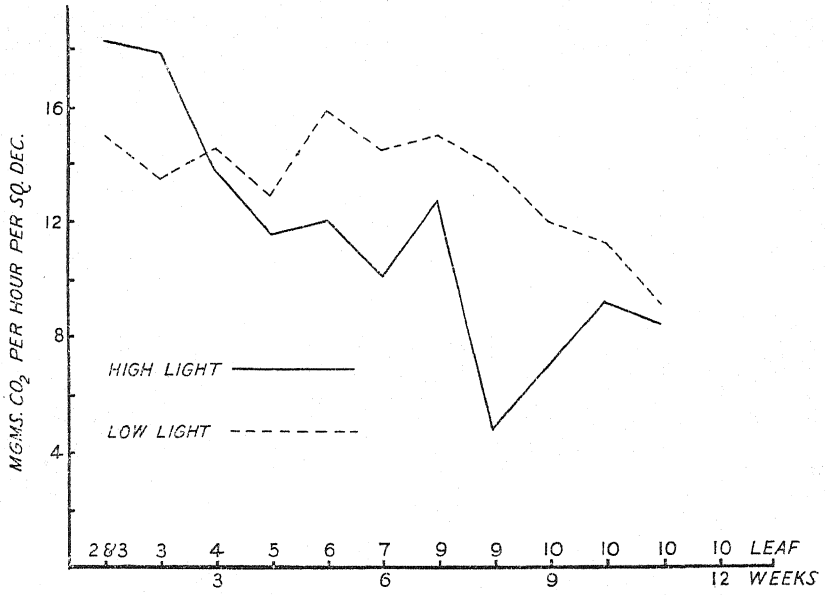


FIG. 11. Graph showing assimilation rate of leaves of nitrate deficient series at high and low intensities of light.

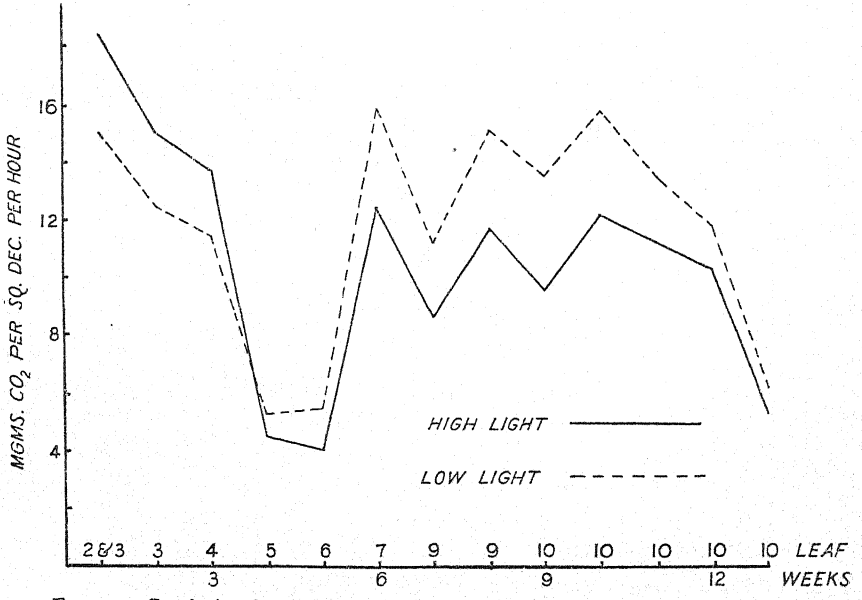


FIG. 12. Graph showing assimilation rate of leaves of potash deficient series at high and low intensities of light.

manured and nitrogen deficient series until the very end, where the values are confined to the last leaf and follow its senescent fall in activity. The phosphate deficient series show a slow but steady decline in assimilation rate at the low light intensity, but to a much less marked extent than at high light intensity.

Analysis of Variance for Assimilation Rate at High Light Intensity.

Mean Value of Assimilation Rate for 11 Values.

Fully manured	13·297	Mean 12·517.
Nitrogen deficient	11·388	
Phosphate deficient	14·395	
Potash deficient	10·988	

	Degrees of Freedom.	Sum of Squares.	Mean Square.	Log Mean Square.	Standard Deviation.
Age	10	338·1926	33·8193	3·52106	—
Manuring	3	85·2007	28·4002	3·34638	—
Remainder	30	215·1559	7·1718	1·97016	2·68 (21·4 %)
Total	43	638·5492	—	—	—

Z (Age : Remainder) = 0·77545 — 1 % point = 0·555, 5 % point = 0·5362
Z (Manuring : Remainder) = 0·68811 — 1 % point = 0·7531

Analysis of Variance for Assimilation Rate at Low Light Intensity.

Mean Value of Assimilation Rate for 11 Values.

Fully manured	1·371	Mean 1·3535.
Nitrogen deficient	1·336	
Phosphate deficient	1·489	
Potash deficient	1·217	

	Degrees of Freedom.	Sum of Squares.	Mean Square.	Log Mean Square.	Standard Deviation.
Age	10	0·5715	0·05715	−2·86208	—
Manuring	3	0·4152	0·13840	−1·97754	—
Remainder	30	1·4514	0·04838	−3·02867	0·2200 (16·3 %)
Total	43	2·4381	—	—	—

Z (Age : Remainder) = 0·0833 — 5 % point = 0·389
Z (Manuring : Remainder) = 0·5256 — 5 % point = 0·536

The series for potash deficiency presents a marked contrast to the others. Here the two curves are very similar in form, and the minor fluctuations agree in their direction in every instance. The marked collapse already referred to in discussing the curves for high assimilation reappear in the curves of low assimilation, and the recovery is shared by both curves to an equal extent. Potash deficiency thus appears to exert a definitely different effect from deficiency in nitrogen and phosphate. In determining the variation in assimilation rates at the two light levels, there are again two main factors at work, namely (1) age effects tending to reduce the assimilation rate with all manurial treatments at high light intensity, but not to such a marked extent at low light intensity, (2) the manurial treat-

ment modifying the age effects in the different series. To disentangle these factors the Analysis of Variance was performed on the two sets of assimilation values, again using the first eleven values alone. The results of the analysis are given in the table opposite.

The results of the analysis throw a very clear light on the interpretation of the curves in Figs. 9–12. The analysis of the data for high light intensity shows that the variance due to age is very significantly greater than that due to experimental error. Z is considerably greater than its value demands for a significance of 100 : 1. The variance due to manuring is also very significant and approaches 100 : 1 probability. In contrast with this the analysis for low light intensity shows that the age effect here is quite insignificant. The manurial effect is, also, insignificant, P being just less than 20 : 1. It may therefore be concluded that the fall in assimilation due to age effects appear only when assimilation rate is studied at high light intensity, whereas when light is 'limiting' the assimilation rate the successive leaves have substantially the same assimilation rate. The standard errors of the mean assimilation rates in the two sets are considerably higher than for respiration rate. The reason for this discrepancy is no doubt the fact that in the remainder variance are included the effects of interaction of age and manuring. The different types of curves obtained in the various deficient series indicates that this interaction is considerable, and hence the experimental error is largely overestimated. Testing the differences of the means of the deficient series with the fully manured, using the standard errors discussed, the results are as follows :

High Light Intensity.

Fully manured – Nitrogen deficient = $+1{\cdot}909 \pm 1{\cdot}143$
" " – Phosphate deficient = $-1{\cdot}098 \pm 1{\cdot}143$
" " – Potash deficient = $+2{\cdot}309 \pm 1{\cdot}143$

Low Light Intensity.

Fully manured – Nitrogen deficient = $+0{\cdot}035 \pm 0{\cdot}094$
" " – Phosphate deficient = $-0{\cdot}118 \pm 0{\cdot}094$
" " – Potash deficient = $+0{\cdot}154 \pm 0{\cdot}094$

For high light intensity the potash series alone gives a significant difference, while for the low light intensity none are significant, but potash approaches the significant value. The Z test, however, leaves little doubt as to the general manurial effect at both light intensities, and it would appear that the series contributing most to the variance is the potash deficient. It is evident that if the differences between the means of the manurial deficient series are taken, phosphate and potash show in both series a significant difference among themselves, thus :

(High Light) Phosphate deficient – Potash deficient = $+3{\cdot}407 \pm 1{\cdot}143$
(Low Light) Phosphate deficient – Potash deficient = $+0{\cdot}272 \pm 0{\cdot}094$

To elucidate the meaning of these results it is necessary to digress briefly into a discussion of the interrelations of factors in assimilation.

Interaction of Factors in Assimilation. Carbon assimilation is controlled by factors which may be distinguished as external and internal. The action of the external factors has been extensively studied, and their interactions on assimilation successfully formulated by Maskell (9). The nature of the internal factors and their mode of control of assimilation is, however, not so clear. The assimilation process certainly takes place at the surface of the chloroplast, and there seems to be general agreement that the whole process consists of at least five stages:

(*a*) The passage of CO_2 from the cell surface to the chloroplast (hydro-diffusion phase).

(*b*) Combination of CO_2 with some component of the chloroplast system, and, finally, with the chlorophyll molecule.

(*c*) Activation of compound molecules by light, chlorophyll possibly acting as photocatalyst, forming some intermediate compound (light reaction).

(*d*) Chemical reaction (catalysed by an enzyme) among the activated molecules leading to the splitting off of oxygen and formation of the first product of photosynthesis (dark reaction).

(*e*) Polymerization of the first product to a final product of assimilation (sugar or starch).

The system here summarized is substantially that formulated by Willstätter and Stoll (17), which, on the whole, seems to be best in accordance with experimental evidence. The whole question of the mechanism of photosynthesis is discussed by Stiles (14), who reviews all the literature to date. The course of photosynthesis may thus be represented by a series of linked processes corresponding with the stages enumerated above. If the external factors are all kept at optimal values, i. e. the natural limit of intensity above which secondary disturbing actions appear (CO_2 narcosis, solarization, temperature time factor), then the rates of each of the linked processes will be determined by the conditions prevailing within the plant (internal factors). The maximal rate of assimilation will thus be determined under optimal external conditions by the internal complex alone. The nature of this complex is largely unknown, although from experimental evidence certain components have been postulated. The process of diffusion into the cell is generally held to be a simple diffusion in imbibed water of the cell-wall and cytoplasm. As will be seen later, this is by no means the only possible or most rapid mode of entry of CO_2, and evidence will be presented lending support to a hypothesis of transport of CO_2 by combination with potassium, in the form of a shifting equilibrium of carbonate and bicarbonate. Potassium may thus be the component of the chloroplast with which combination of CO_2 first occurs. The velocity of the photo-

chemical process will be determined by four factors: concentration of CO_2 (or HCO_3 ion), intensity of light, number of chlorophyll molecules available, and a proportionality factor. The work of Willstätter indicates that the velocity of the reaction is not proportional alone to the mass of chlorophyll present, so that the assimilation number varies within wide limits. Briggs (2), moreover, has claimed that by maintaining the amount of chlorophyll at a low constant level the assimilation rate changed with the age of the leaf, even when light is 'limiting' the assimilation process, in which case there is no question of the efficiency of the dark reaction limiting the rate of the whole process. The evidence for this conclusion of Briggs's is indeed very slender, being based, in fact, on a single experiment with a normal leaf as compared with partially green leaves of *Phaseolus* (Briggs's paper, Table B and Appendix Table VI). Comparison of the figures in Table B of Briggs's paper shows that considerable variation in assimilation rate may occur among individual leaves apart from the stage of greening reached, both at high and low light intensities. If, however, this result is accepted as well established, it is clear that the proportionality factor varies even though the chlorophyll content remains constant. If a curve were constructed showing the relation between assimilation rate and light intensity at low light intensity, where the photochemical reaction controls the rate of the whole process, the resulting curve would be a straight line, and the tangent of slope of this line would be a measure of the proportionality factor. If Briggs's contention is true, the slope of this line would vary with age of otherwise similar leaves, and this slope would be a measure of the efficiency of the photochemical system. Briggs's 'reactive chloroplast surface' is, in fact, nothing but this measure of efficiency, which cannot be estimated except from the slope of the assimilation-light-intensity curve, and his interpretation of it would seem to be purely arbitrary. It is true that a term with the dimensions of area must enter into the function of assimilation rate, but the conclusion that the changing proportionality factor is due to change in this area is unwarranted without further assumptions. The light-intensity-assimilation rate curve for two leaves of different ages (even when light is the limiting factor) may thus be represented formally, as in Fig. 13, A.

The same diagram would equally represent the curves for two different plants varying in reactive chloroplast surface, or in efficiency of photochemical activity. The seasonal variation in assimilation rate for Cherry laurel, even when light limited, led Blackman and Matthei to postulate a 'protoplasmic factor' in assimilation. It is not clear why Briggs (2, p. 257) limits the application of this term to the chemical phase. Applied to both phases it would seem to be a measure of the proportionality factor similar to 'reactive chloroplast surface', both being terms describing the experimentally determined differences in photosynthetic efficiency.

The chemical or dark reaction will depend in rate on four factors: concentration of molecules concerned (activated molecules from the photochemical reaction), temperature, concentration of the final product (first product of photosynthesis), and the velocity constant of reaction. If it is assumed that the final product is removed rapidly when compared with the

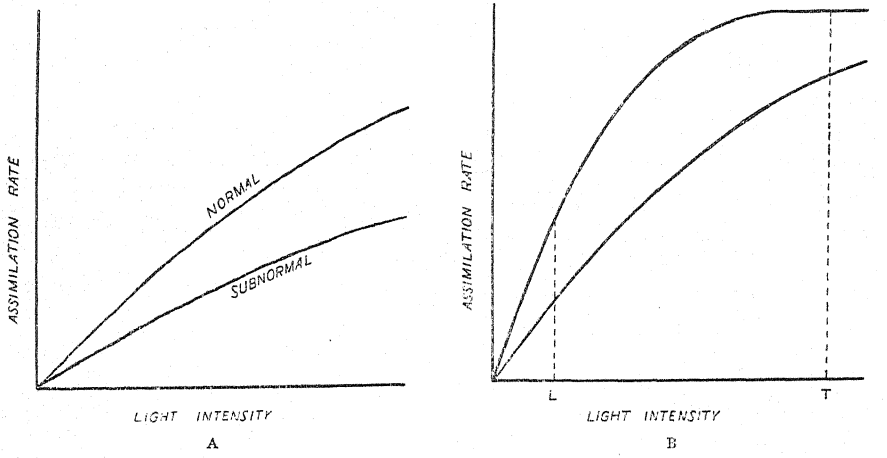


FIG. 13, A and B. For description see text.

reaction under consideration, the rate of the process will be determined solely by the concentration of activated molecules from the photochemical phase and the velocity constant. As the temperature is raised a velocity in the dark phase will finally be reached, at which the number of activated molecules interacting equals the number formed by the photochemical reaction. At this temperature assimilation is light limited. It is clear that with the above system, as postulated by Briggs, the temperature limited values of assimilation rate must be completely determined by the concentration of activated molecules, and hence by the velocity of the photochemical reaction, and therefore with two leaves differing in rate of photochemical reaction the light limited as well as temperature limited values of assimilation rate must be directly proportional to these rates. Since the proportionality factor of photochemical reaction has been called 'reactive chloroplast surface', it follows from Briggs's assumption that 'any change in the amount of reactive surface per unit of leaf will necessitate a change in the photosynthetic activity both when light and when temperature is limiting'. It seems possible, however, that the 'protoplasmic factor' is directly concerned with the dark phase (in fact, Briggs limits the concept to activity in this phase), and, if, as Willstätter assumes, the dark phase is activated by an enzyme, then the velocity of reaction in the chemical phase is not only proportional to the concentration of activated molecules and temperature but to the concentration of enzyme present, and the pro-

portionality factor will now be no longer determined by the 'reactive surface' in the photochemical phase. That such an activation occurs is clear from Willstätter and Stoll's experiments with green and yellow leaves (17, Figs. 7 and 8, p. 149). The results can be formally represented, as in Fig. 13, B.

The ratio of assimilation rates at the light limited values (L) to that at temperature limited (T) is widely different, and, moreover, varies all over the range of light intensities. That Willstätter's values for 25° C. in Figs. 7–8 are temperature limited at intensity I is clear by comparison with his Figs. 9–10 where assimilation rate is still rising at 30° C. Figs. 9–10 show clearly that the temperature coefficients differ in the two cases (although light is the limiting factor, as can be seen from the values in Fig. 9 compared with those of Fig. 7 with double light intensity), and, as discussed above, Briggs's assumptions would lead to an identical temperature coefficient in each case. It would seem, therefore, that the 'protoplasmic factor' is of a more complex nature than is envisaged by Briggs's 'reactive surface', and that 'doubling the "reactive surface" should double the activity when temperature is limiting' is, indeed, inherent in the assumptions but not substantiated by experimental fact.

From the foregoing discussion it is evident that it is not necessary to anticipate that the ratio of light-limited and temperature-limited rates should be constant, or that in leaves from plants deficiently manured the subnormality in the light limited rate should be the same as that in the temperature limited. On the contrary, manurial deficiency may act specifically on the efficiencies of the photochemical and dark reactions leading to diverse types of 'subnormality'. Before going on to discuss these specific deficiency effects it is well to be clear as to the meaning of the term 'subnormality'. As has been shown above from the Analysis of Variance, there is in all the series investigated a fall of assimilation rate with age of leaf. The assimilation rate of the leaf depends, therefore, on the age of the plant at the time at which the leaf develops, as well as on the age of the individual leaf. Later formed leaves therefore display 'subnormality' with respect to the earlier leaves, and, as has been shown in the case of the fully manured plants, this subnormality is confined to the rate of assimilation at high light intensity. It is presumably the dark reaction which is affected by the age of the plant as a whole, and the difference may be attributed to differences in enzymatic equipment. A similar fall in respiration has also been established, and equally indicates a fall in activity of enzymes, since the carbohydrate content of the whole plant is presumably rising, and hence fall in respiration cannot be put down to fall in concentration of respirable material. This effect of age confined to the high light values of assimilation is seen equally clearly in Briggs's experiments with *Phaseolus* (his Table VII) in which, for the fully manured plants, the light limited values for leaves of different ages is constant (2·57, 2·56, 2·79 mg.),

while the temperature limited values fall rapidly (5·71, 3·95, 3·35).[1] In dealing with the problem of subnormality this effect must be borne in mind, and leaves of similar morphological age alone must be compared. The method of Analysis of Variance allows this effect to be disentangled from the effect due to manuring, and, as has already been shown, the manurial effect is significant at high light, and almost so at low light, intensity; it would certainly be so if the variance due to the interactions of age and treatment were deducted from that given for the Remainder.

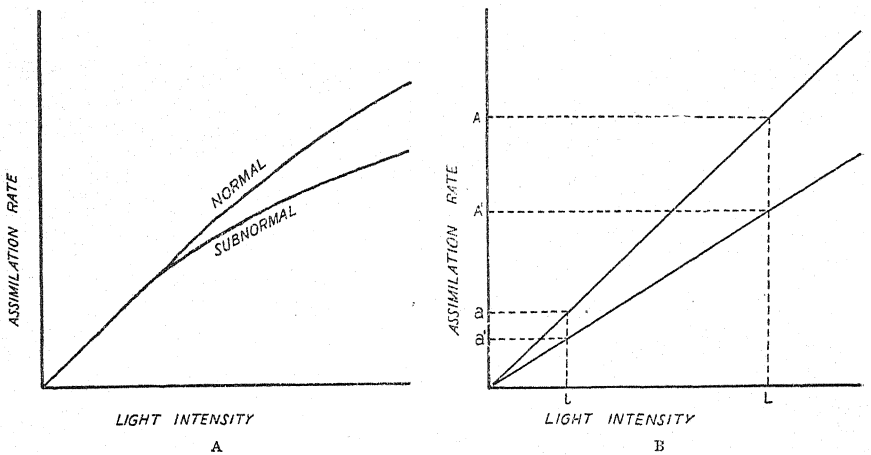


FIG. 14, A and B. For description see text.

The two types of subnormality may be represented formally by the Figs. 13, A, and 14, A, the former showing the typical age effect, as found in the fully manured plants, and Fig. 14, A, the type found in potash and to a less extent in phosphate starved plants.

The experimental results obtained in this investigation will now be examined with a view to establishing the type of subnormality found in the various deficient series.

Specific Manurial Effects on Subnormality. Since only two points on the curves at the two light intensities are available, it is only possible to elucidate the types of assimilation curves for successive leaves by indirect means. If the curves are of type 14, A, however, the assimilation rate at high light intensity should be independent of the low light rate, i. e. if the mean of the rates at high light intensity are taken, then a positive deviation of the value of a particular leaf from the mean should not necessarily be associated with a value above the mean at low light intensity for the same leaf. Correlation coefficients were therefore calculated between maximum assimilation and minimum assimilation rates for pairs of values of all the leaves investigated.

[1] The value 3·35 has been substituted for 3·95 given in the table which is obviously incorrect as can be seen from Table V where 3·35 is given, and confirmed by the per cent. values in Table VII.

The results are given below:

Correlation Coefficient of Maximum Assimilation and Minimum Assimilation Rates.

Fully manured	$r = -0.111$
Nitrogen deficient	$r = +0.403$
Phosphate deficient	$r = +0.742$
Potash deficient	$r = +0.776$

The values for phosphate and potash deficiences are highly significant ($P > 100:1$), while for fully manured and nitrogen deficiency the values obtained are quite insignificant.

An objection may be raised at this point on the ground that the assimilation rate at low light intensity has been calculated from the respiration rates and the known light intensities at the compensation point, assuming direct proportionality between light intensity and assimilation rate at this low light intensity. To test whether such proportionality exists the correlations between respiration rates (uncorrected for temperature) and the light values at the compensation point have been calculated with the following results:

Correlation Between Respiration Rate and Light Value at the Compensation Point.

Fully manured	$r = +0.928$
Nitrogen deficient	$r = +0.875$
Phosphate deficient	$r = +0.955$
Potash deficient	$r = +0.005$

The uniformly highly significant ($P > 100:1$) and high values of the correlation coefficient in all cases except the potash series, indicates clearly the proportionality existing between light value and assimilation rate, and the near approach to linearity of the assimilation rate curves at low light intensity. These correlation coefficients are not significantly different when the effect of time is eliminated. In all cases but that of the potash series the reduction of assimilation rate to a uniform light value (the mean of the values at the compensation points) by simple proportion would therefore introduce no significant errors into the estimates of low assimilation rates utilized for correlations with high assimilation rates given above.

The approach to rectilinearity of the assimilation curve at low light intensity may be seen from the graphical representation in the phosphate deficient series given in Fig. 15, together with the regression line calculated from the data. In the case of the potash deficient series the low value of the correlation coefficient indicates that even round about the compensation point the assimilation rate is not directly proportional to light intensity. In this case the justification of the reduction of assimilation rates to a uniform light intensity by simple proportion rests on another consideration. It has already been shown in Fig. 12 that the fluctuations in assimilation

rate at high and low light intensities are closely similar, in fact the ratio $\frac{\text{assimilation at high light}}{\text{assimilation at low light}}$ approaches a constant value. The low correlation between respiration rate and light intensity at the compensation point, already referred to above, is, however, negligibly small, indicating that the curves of assimilation rate and light intensity for successive leaves in no part overlap, but have different slopes even near the origin; in fact, are of the form schematically represented in Fig. 14 B.

If a, A represent the assimilation rates at two light intensities l, L respectively for leaves of one age and a', A' represent the assimilation rates for leaves of a different age at the same light intensity, then $a/A = a'/A' = l/L$. In these experiments L is the high light intensity which is kept constant, l the low light intensity which is the light value at the compensation point, while a is the respiration rate uncorrected for temperature, and A the observed assimilation rate at high light intensity. The theoretical considerations just discussed may therefore be tested by calculating the correlation coefficient of $\frac{\text{Respiration}}{\text{Max. assimilation}}$ and the light value at the compensation point. This has been done for each set of experimental values, with the following result:

Correlation $\frac{\text{Respiration uncorrected for temp.}}{\text{Max. assimilation rate}}$ and light value at compensation point.

Fully manured	$r = +0{\cdot}151$
Nitrogen deficient	$r = +0{\cdot}210$
Phosphate deficient	$r = +0{\cdot}728$
Potash deficient	$r = +0{\cdot}900$

Both the phosphate and potash deficient series give highly significant correlation coefficients. It may therefore be assumed that for any single leaf in the case of the potash deficient series no serious errors will be introduced in reducing all the low assimilation rates to a mean light intensity by simple proportion. It is therefore clear that the method used for reducing the minimum assimilation rates to the mean light value at the compensation point is amply justified. The correlations for the phosphate deficient series call for further comment. It has been shown above that the correlation coefficient of respiration rate (uncorrected for temperature) and the light value at the compensation point is highly significant ($r = +0{\cdot}955$), and further that the correlation coefficient $\frac{\text{Respiration uncorrected for temp.}}{\text{Max. assimilation rate}}$ and light value at the cp. point is also highly significant ($r = +0{\cdot}728$), thereby contrasting with the potash deficient series in which the first correlation is insignificant ($r = +0{\cdot}005$), while the second is very highly significant ($r = +0{\cdot}900$). Thus for phosphate deficiency the respiration rate is almost proportional to the light value at the compensation point (Fig. 15), and,

further, the ratio of respiration to maximum assimilation is also approaching proportionality with the light value at compensation point. In other words, R/L approaches constancy, also $R/L \times A$ approaches constancy (R = respiration rate uncorrected for temp., L = light value at c.p.). Now R/L

$$= \frac{\text{assimilation rate reduced to mean light value at the c.p. } (a)}{\text{mean light value at compensation point}}$$

therefore for the second relation to hold a/A must approach a constant value,

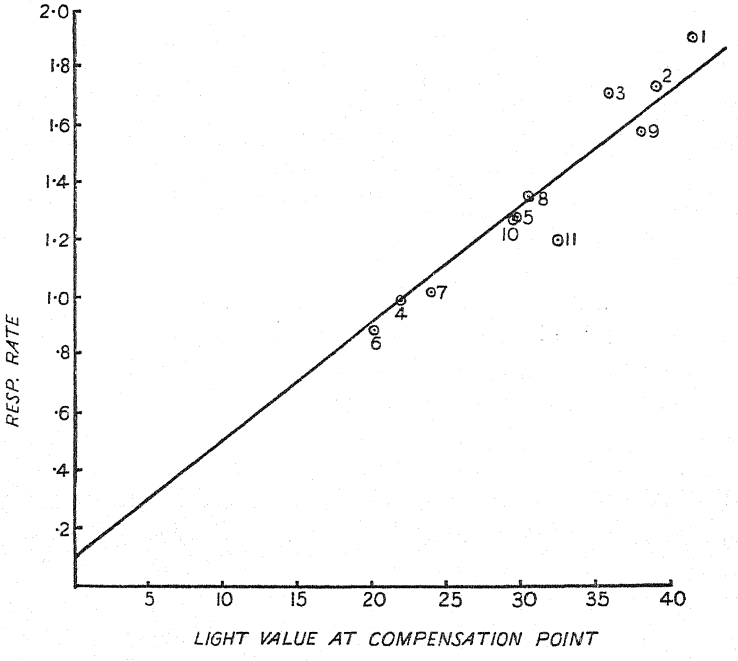


FIG. 15. Graph showing respiration rates and light values at the corresponding compensation points.

in other words, max. and min. assimilation must be highly correlated. Reference to these correlation values given above confirms this, and, further, the value of a ($R/L \times$ constant) is remarkably uniform in this series. The correlation value of a and A indicates that any changes in a are associated with changes of A in the same direction, and a reference to Fig. 10 shows that the curve for the two assimilation rates fall together. If this drift is the main cause of the high association, it might be expected that the fall in low assimilation rate curve with time should be significant. In fact, the correlation values of low assimilation rate and time for the phosphate series is highly significant ($r = -0.796$), whereas for the other series it is quite insignificant (F. M. + 0·276, N. def. − 0·502, K. def. + 0·238). It is thus evident that the age effect in the case of phosphate deficiency affects

the assimilation rate even at low light intensity. Since this ageing effect is in part due to falling P_2O_5 concentration in the leaves, it is seen that as the concentration falls the assimilation falls even at low light intensity. Further, the highly significant correlation of respiration rate and maximum assimilation ($r = +0{\cdot}681$) indicates that phosphate deficiency affects respiration as well as assimilation at both high and low light intensity. The evidence suggests that phosphate is concerned in maintaining the level of assimilation efficiency (active chloroplast surface) as well as respiratory activity.

The effects of manurial deficiency established above are summarized in Table IV (p. 136).

From the table it is clear that the effect of each manurial constituent is specific, and that only in the case of potash deficiency is the assimilation rate subnormal both at high and low light intensities.

The Effect of Potash Deficiency on Respiration and Assimilation Rate. Potash deficiency seems to have an effect differing from that of deficiency in nitrogen and phosphate, in that the assimilation is subnormal both at high and low light intensity. It is already known from previous work (unpublished data) that in the cases of plants grown either with full or deficient manuring of nitrogen and phosphorus the percentage of these elements in the successive leaves decreases. This question and its bearing on growth processes will be discussed in a later paper. At present it may suffice to state that this effect is invariably seen whatever the level of manuring applied, and is a direct consequence of the fact that nitrogen and phosphate control the tillering rate, and that tillering proceeds until internal starvation supervenes. In conditions of nitrogen and phosphate deficiency tillering is slower and is arrested at an earlier stage, so that the internal concentrations of nitrogen and phosphate are automatically adjusted, and internal starvation is no more acute than it eventually becomes in the highly manured plants. Potash deficiency, however, is found to have a much less marked effect on tillering, which proceeds at nearly the same rate as in fully manured plants, with the consequence that internal starvation for potash becomes very acute. This is reflected in the characteristic behaviour of the plant, whose leaves become increasingly yellow, a stage corresponding in time with the rapid collapse in assimilation rate; tillering, however, still proceeds, even when the plants apparently are about to die. Rapid death of tillers now occurs, and death also on the lower leaves on the tillers. After this the new leaves which appear on surviving tillers are darker green, and from this time onwards death of leaves keeps pace with new development, so that at most two living leaves are found on each tiller. This recovery of the plants coincides with the recovery in assimilation rate which becomes nearly normal. It would seem probable that the death of leaves and tillers sets free the potash which is then translocated to the developing leaf. Chemical analyses of the

single leaves used in this investigation were therefore undertaken[1] and the results are presented in Table V.

TABLE V.

Leaf No.	Potash Deficient.			Fully Manured.		
	% K_2O in Dry Wt.	K_2O (mg.) per sq. dm.	$\frac{K_2O}{\text{Water Content.}}$	% K_2O in Dry Wt.	K_2O (mg.) per sq. dm.	$\frac{K_2O}{\text{Water Content.}}$
2 and 3	1·03	3·26	1·36	2·43	9·39	4·66
3	0·69	2·52	1·11	2·03	8·52	3·89
4	0·65	2·19	0·94	2·35	7·70	3·76
5	(0·67)	(1·98)	(0·75)	0·97	3·32	1·42
6	0·41	1·12	0·56	2·06	6·44	3·30
7	0·37	0·95	0·54	1·08	4·24	3·00
9	0·29	0·76	0.49	1·90	7·95	5·43
9	0·40	1·03	0·73	1·17	4·34	3·26
10	0·30	0·96	0·90	2·21	8·76	8·68
10	0·40	1·36	1·18	2·40	9·25	9·29
10	0·54	1·63	1·56	2·00	7·41	7·78
10	0·27	0·87	0·72	1·67	5·76	8·25
10	0·29	0·99	0·84	—	—	—

The figures in the above table show the large differences found in the potash content of leaves from deficiently manured, as compared with the fully manured, plants. Each series shows also a rapid decline in the potash content in the early stages of growth while tillering is proceeding, reaching a minimum, and subsequent recovery, as the potash, liberated by the dying parts of the plants, is translocated up to the later formed leaves. Except for the fifth leaf in the fully manured series, which seems quite erratic, the percentage of potash in the dry weight for this series, remains higher than for any value in the deficiency series. The weight of potash divided by the weight of water in the leaf is also given in the table. This figure gives a measure of the concentration of potash in the leaf, assuming that all the potash is present in solution. The values of this ratio, together with respiration rate and weight per unit area of leaf, are given in graphical form in Fig. 16. The correlation between the three curves is very striking. There would seem here to be a contradiction with the findings given earlier in the paper, where it was shown that respiration in general was higher in the potash deficiency series, whereas here it is seen that as the potash content of the leaf changes, the respiration varies in the same direction. The relation is, however, entirely spurious as the following correlation coefficients show.

Correlation potash concentration (x) and respiration rate (y) $\quad r_{xy} = + 0{\cdot}509$

Correlation potash concentration (x) and dry weight per unit area of leaf (z) $\quad r_{xz} = + 0{\cdot}521$

[1] The authors wish to record their thanks to Dr. Janet W. Brown for performing these difficult analyses.

Correlation respiration (y) and dry weight per unit area (z)	$r_{yz} = +0{\cdot}859$
Partial correlation of potash concentration (x) and respiration (y) eliminating (z)	$r_{xy \cdot z} = +0{\cdot}142$

It is apparent that if the effect of weight per unit area is eliminated, i. e. taking the hypothetical case of leaves of equal weight per unit area but of varying potash content, the potash concentration is without significant effect on the respiration rate. The relationship between weight of unit area of leaf and respiration rate is itself interesting. Since weight per unit area depends on the mass of dead tissue and the mass of cell contents, and the former can have no conceivable effect on respiration, it would seem that the major part of the changing weight per unit area is due to the mass of cell content. Presumably then in this case the weight per unit area is a measure of the mass of protoplasm present, and the effect of decrease in nitrogen in lowering respiration rate is presumably due to the same effect of mass of protoplasm working in the reverse direction. The falling respiration of the whole plant (7), which may be correlated with falling nitrogen content (12), is presumably due also to the falling mass of protoplasm per unit dry weight of the plant. A similar high correlation between respiration and weight of unit area of leaf is seen in the fully manured series ($r_{yz} = +0{\cdot}545$), whereas the correlation between respiration and potash content is here quite insignificant ($r_{xy} = -0{\cdot}161$). It would seem, therefore, that potash concentration has no direct effect on respiration rate, but indirectly affects it by determining the level at which other constituents of the cell are maintained.

The correlation coefficient of potash concentration and assimilation rate at high and low light intensities, respectively, eliminating the effect of time, are $r = +0{\cdot}591$ for high intensity, $r = +0{\cdot}374$ for low light intensity. The first of these values is significant. The original rapid fall in assimilation rate corresponds with the initial rapid fall, and the recovery is accompanied by a rise in potash concentration. The value for the seventh leaf is, however, greater than for the fully manured, while the potash concentration continues to fall for two further weeks before the rise begins. The high correlation between maximum and minimum assimilation rates indicates, however, that some factor is at work determining the efficiency of the photosynthetic process. It is difficult to correlate this with anything but the potash content of leaves. The initial fall in assimilation rate is equally marked at low as at high light intensity, thereby contrasting with the course of assimilation in any other series, in which the assimilation at low light remains almost constant during the initial rapid fall at high light intensity. The possibility presents itself that potash concentration plays a part in the diffusion of CO_2 into the cell up to the chloroplast surface. Some evidence for this is found in the work of Willstätter, who found that

dry leaf material of certain plants will readily take up CO_2, an effect shown by Spoehr and McGee (13) to be associated with the ash content of the leaf, and due to the formation of bicarbonates. It is not suggested that

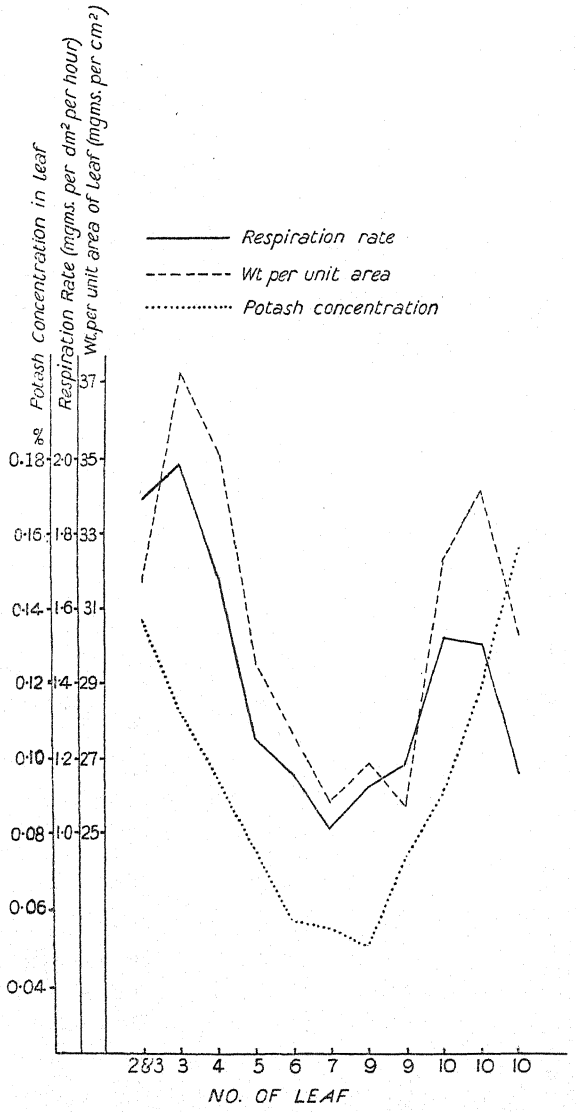


FIG. 16. Graph showing for successive leaves of the potash concentration, the respiration rate, and the weight per cent. area of leaf.

potassium alone could subserve this role, in fact either sodium or calcium are capable of forming bicarbonate. Potassium, however, has two peculiarities which would particularly lend themselves, in that (1) the percentage of potassium in the total ash is very high (as much as 50 per cent. in the

leaves analysed, whereas sodium only occurs in small percentage in most leaves), and (2) potassium is apparently very mobile within the cell, and may be washed out of living cells, indicating that the cytoplasm is extremely permeable to potassium ions. Further work is now in hand which aims at elucidating more clearly the relation of potassium manuring to assimilatory capacity of barley plants, and hence it is not intended without further substantiation to labour this suggestion. In these experiments external CO_2 was kept so high (4–5 per cent.) that gaseous diffusion into the stomata and up to the external cell-wall may be considered to have introduced a very small resistance into the whole path. The concentration at the intercellular wall surfaces may therefore be considered constant, while the concentration at the chloroplast surface is determined by the light intensity leading to disappearance of CO_2 at this point. The rate of passage of CO_2 will thus be determined by the difference between the concentration levels at the surface of the cell and the chloroplast, divided by a resistance factor, itself determined in magnitude by the concentration of the potassium ions present. If light is the 'limiting factor' the falling concentration from the surface of the cell to the chloroplast surface will be proportional to the light intensity, and for leaves differing in potassium concentration the rate of passage of CO_2 will tend to be proportional to the potassium concentration if this is low. With increasing light intensity, therefore, the assimilation rate would be subnormal in the 'low potash' leaf all over the range of light intensity, and, moreover, the subnormality at high and low light intensity would be proportional. A further deduction would follow, namely, that for low potash leaves for a given light intensity temperature limitation would occur at a lower temperature than for a high potash leaf, or, in other words, the temperature coefficient of assimilation in the higher temperature regions would be *higher* for high potash than for low potash leaf. From work now in hand these deductions will be capable of experimental test.

General Discussion.

In reviewing the results put forward in this paper two considerations must be borne in mind: firstly, deficiency in nitrogen and phosphate was less acute than in potash; secondly, it is not certain whether at high light intensity the assimilation was temperature-limited. Dealing with the first consideration it may be stated that this difference in manuring was deliberate, since nitrogen and, to a less extent, phosphate have been found from previous work to exercise a very marked controlling influence on growth, whereas equivalent doses of potash affect growth to a less marked extent. It seemed desirable to avoid carrying the level of manuring so low that very abnormal plants would be obtained, since it would then be difficult to compare the direct measurements of assimilation rate with those of net

assimilation rate obtained from growth data in previous years. The fact remains that all the typical specific symptoms of starvation were very marked in the plants used; thus the nitrogen starved plants had many fewer tillers than the fully manured, the leaves were a much lighter green and differed characteristically, as was shown in section three of this paper. The phosphate starved also had fewer tillers, and the leaves after the third showed the characteristic reddening associated with phosphate starvation, as well as more pointed and rolled type of leaf. In the fourth determination of this series the green base of the leaf was used, although the upper part of the leaf was orange in colour and almost devoid of chlorophyll. In spite of this the assimilation rate of the green part was apparently unaffected, and not until a fortnight later, when leaves wholly green were used, did the photosynthesis reach a minimum. The potash starved plants also presented a very characteristic appearance, having very yellow thin broad leaves, marked inhibition of stem growth, and completely sterile ears. It is remarkable, therefore, that the deficiency effects obtained differ so markedly from Briggs's findings with *Phaseolus*, for in the latter case the leaves are expressly stated to have been normal in appearance, except for the iron starved, which were chlorotic, and the nitrogen starved plants, for which, however, no assimilation data appear.

Real assimilation rates were obtained by Briggs by adding to the apparent assimilation a 'value well established for normal leaves of *Phaseolus*, namely, 1·5 cc. per gramme dry weight per hour at 15° C.'; without experimental evidence it would seem unlikely that the cotyledons and first pinnate leaves of the plants used by Briggs actually had the same respiration rate. In any case Briggs ruled out entirely the possibility that manurial deficiency had an effect on respiration rate, an effect which the present series of experiments has shown to be considerable, i. e. as much as 60 per cent. in potash starved on leaf area basis, and up to 95 per cent. on dry weight basis above the fully manured. Regarding Briggs's results collectively they appear to show subnormality due to manuring, and an Analysis of Variance performed on his data confirms the impression. In order to obtain a symmetrical table the two phosphate results were omitted, leaving twelve available results. For the low light intensity the variance due to manuring ($Z = 0{\cdot}5930$) was found to be quite insignificant (5 per cent. point = 0·7798), while for the high light intensity a very significant effect of manuring is apparent ($P > 100 : 1$). The evidence from Briggs's data thus indicates a real subnormality at high light intensity, but no such significant effect at low light intensity. Further, the age effect is very highly significant at the high light, and quite insignificant at the low light, intensity ($Z = 0{\cdot}413$, 5 per cent. point = 0·819). Briggs's results therefore confirm those found in these experiments, in so far as the age effect is much more marked at high light intensity where also subnormality preponderates.

When the individual results are compared it is clear that the lack of iron has greatly affected the assimilation rates at both light intensities, and there is a steady decrease in rate with successive leaves. As far as the two series comparable with the present experiments are concerned the evidence is not so clear. In the case of phosphate deficiency the first simple leaves showed no decrease in assimilation rate, and the first pinnate leaves gave rates of 84·8 per cent. and 82·5 per cent. of the values obtained from a similar single experiment with fully manured plants. As a third experiment is not reported, the evidence would depend on a single comparison, and amount to a reduction of approximately 16 per cent. In the case of potash deficiency the first simple leaf showed a slight reduction (87 per cent. and 96 per cent.) below the fully manured, but the first pinnate leaf (when shortage was presumably more acute) gives values identical with the fully manured. The onus of proof thus rests on the third experiment. In Briggs's Table VII (Summary of Results) the values at high and low light intensity are 67 per cent. and 83 per cent. of those given for fully manured plants. In Table V, however, a third value for yet lower light intensity is given for leaves of both series, and here the potash starved value is 80 per cent. of the fully manured. These three values (80 per cent., 67 per cent., 83 per cent.) should, if Briggs's theory is correct, be identical, and the occurrence of an intermediate value differing so markedly from the two others, indicates that possible errors in technique are involved. The difference in this series of values is of the same order as between the manurial deficiency series and the fully manured control. Further, another source of variation, namely, that due to individual differences in plants similarly treated, has not been considered, and as the present author's experiments show these may be very considerable. Analysis of Variance on Briggs's data has shown that for the high light values the standard deviation after eliminating known sources of variation is 10 per cent., and for the low, 22 per cent. It is thus clear that, from such few determinations as Briggs made, it would be rash to conclude that as far as phosphate and potash starvation are concerned there is any real subnormality at all due to manurial deficiency, apart from the possible relative effects on light-limited and temperature-limited assimilation rates.

In the experiments reported in this paper it is doubtful if the assimilation rates at the high light intensity were temperature limited. As previously stated the light intensity used was 5,000 lux, while Briggs claims to have worked at 36,000 lux. It should be made clear, however, that the light intensity (5,000 lux) here used was experimentally determined; had the intensity been calculated merely from the candle power of the lamps, it would have been rated at a much higher value. Thus the three half-watt lamps used totalled 450 watts, and were at a distance of 8 in. from the leaf chamber; this, assuming as low an efficiency as one candle per watt,

would give a *calculated* value of 10,000 lux. It is therefore evident that calculated values of light intensity are suspect. Willstätter states that he calculated the light intensity, while Briggs gives no clue as to how the light intensities in his experiment were estimated. Even though the high light values were not temperature limited, it would be difficult to reconcile the results obtained in the present experiments with Briggs's contention of the 'active chloroplast surface'. It would appear that even the efficiency of light-limited assimilation varies with light intensity. The simplest explanation would be that with rising light intensity the assimilation rate is more and more controlled by the rate of the dark reaction, a result which is easily explicable on the assumption of a reversible reaction in the photochemical phase. Under these circumstances the concentration of the intermediate compounds (which is the substrate of the dark reaction) would depend on the relative velocities of both reactions. With rising light intensity the concentration of the intermediate compound would tend to rise, which would result in increase in rate of the dark reaction, until the rate of formation of the intermediate compound just equalled the rate of removal by the dark reaction. In such a system both reactions would control the rate of assimilation at all light intensities, and the 'partial limitation' would depend on the relative velocities of two reactions. That such a reversible reaction is concerned is indicated by Warburg's experiments with intermittent light (16).

Finally, it may be pointed out that the results here recorded corroborate previous work done by one of the present authors (5), in which net assimilation rates in the early stages of the life cycle up to maximum leaf area are shown to be independent of the level of nitrogen manuring maintained. In this case assimilation is limited by CO_2 supply, since the plants were grown in the open air, and, apparently, under these circumstances the assimilation rate remains constant and is unaffected by age, just as was found for nitrogen deficient and fully manured plants in the present experiments when light was limiting. In both these cases the assimilation is controlled almost completely by the level of the external factors, the internal factors (efficiencies of the light and dark reaction) being sufficiently high to maintain assimilation rate at a value determined by the level of the external factors. Unpublished work with phosphate manuring has shown that the net assimilation rate under outdoor conditions falls continuously if phosphate level is low, so that, as in the present experiment, phosphate shortage would appear to lead to 'subnormality' even when assimilation rate is limited by external factors (light or CO_2).

In conclusion the authors wish to thank Sir E. J. Russell for granting facilities for the work to be performed at Rothamsted, and Professor V. H. Blackman for his ready interest and helpful criticism.

SUMMARY.

1. The use of the katharometer for the measurement of respiration and assimilation rates of leaves is discussed; methods are indicated of overcoming some of the difficulties in its use.

2. The effect of deficiency in nitrogen, phosphorus, and potash on water content and weight per unit area of successive leaves of barley, as compared with those of fully manured plants, are studied; they lead to the conclusion that leaf area is a better basis than is dry weight for the expression of water content of leaves.

3. Respiration rates for successive leaves of nitrogen, phosphorus, and potash deficient plants as compared with fully manured plants are given. In all the deficient series, the rate of respiration falls to a minimum with a subsequent rise; while in the case of the fully manured plants the rate falls rapidly at first, becoming constant later. Nitrogen starved plants are shown to have a consistently lower respiration rate than fully manured, potash a consistently higher rate, and phosphate to be unaffected. The differences found are as follows:

	Differences of Means.
Fully manured – Nitrogen deficient	$+0{\cdot}489 \pm 0{\cdot}127$
Fully manured – Phosphate deficient	$-0{\cdot}106 \pm 0{\cdot}127$
Fully manured – Potash deficient	$-1{\cdot}574 \pm 0{\cdot}127$

Analysis of Variance shows that the effect of age of plant and manurial deficiency are both very significant ($P > 100 : 1$).

4. Assimilation rates at known high and low light intensities are given for successive leaves of fully manured plants, and also for each of the deficient series.

It is shown that changes in assimilation rate are due to the action of two factors, namely, age of plant and manurial deficiency. By Analysis of Variance the following facts were established:

(i) At low light intensity the effect of age is quite insignificant, but the manurial effect is almost significant, due predominantly to the value of the potash deficient series.

(ii) At high light intensity the effect of age is very highly significant ($P > 100 : 1$), and the manurial effect is also very significant.

The bearing of these results on the nature of the 'internal factor' in photosynthesis is discussed. It is shown that two types of subnormality occur, namely, that due to age and that due to manurial deficiency. Subnormality of later formed leaves, as compared with earlier, is found in all the series.

5. Subnormality due to manurial deficiency is found to be specific in effect for the various constituents. The results obtained may be summarized thus:

	Respiration.	Assimilation.	
		Low Light Intensity.	High Light Intensity.
Fully manured	Normal	Unaffected by age of plant	Falling with age of plant.
Nitrogen deficient	*Subnormal*	Normal; unaffected by age of plant	Subnormal; falling with age of plant
Phosphate deficient	Normal	Slightly supernormal. *Falling with age*	Slightly supernormal. Falling with age.
Potash deficient	*Supernormal*	*Subnormal*	*Subnormal*

6. The subnormality due to potash deficiency is further discussed, and its theoretical bearing indicated.

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The Effect of Small Electric Currents on the Assimilation of Elodea canadensis.

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With one Figure in the Text.

1. INTRODUCTION.

IN 1896 Thouvenin (6) carried out some experiments on the effect of electric currents on water plants; he found the rate of assimilation in *Elodea canadensis* increased under the influence of the current. He took the change in rate of the bubbles given off from the cut end of the stem, as a measure of the increase or decrease of the assimilation. His results are, however, not very reliable because he attached his electrodes directly to the plant and did not take into account any injury which the plant may thereby have sustained. Also it is highly probable that the bubbles which he thought were the result of assimilation were in part gases produced by electrolysis. Another criticism that may be made is that the temperature was allowed to rise 5° to 7° C. during the experiments; this alone would account for any increase in the rate of bubbling.

Some years later, in 1907, Pollacci (5), experimenting on leaves through which electric currents were passed, found that these gave a higher rate of assimilation as compared with non-electrified leaves; he also attached the electrodes directly to the leaf, and his results are of very doubtful value.

In 1908 Koltonski (4) carried out some experiments similar to those of Thouvenin, and investigated the effect of electric currents on the assimilation of *Elodea*. When the plant was lying across the current he found that for small currents from 0·608 to 0·85 milliamps per cm.^{2}, the bubbling rate increased, but for higher currents it decreased. He carried out other experiments in which the plant lay parallel to the current; the lowest current he used in this case being 0·16 milliamp per cm.^{2}, but he obtained only negative results. He found that when the direction of the electric current was through the plant from tip to base, the bubbling rate decreased more than when the current was in the opposite direction. In addition to

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these experiments he carried out others in which he attached the electrodes directly to the plant, but for reasons already stated results by this method are unreliable. Koltonski concluded from his experiments that small electric currents increased the rate of assimilation.

Of recent years Blackman (1, 2 and 3) and his co-workers have carried out experiments on the effect of high tension electric discharges on plants, and have obtained evidence that the yield of cereals can be increased both in the field and in pot-culture. It seemed, therefore, timely to re-investigate the question of the effect of small currents on the rate of assimilation of *Elodea*.

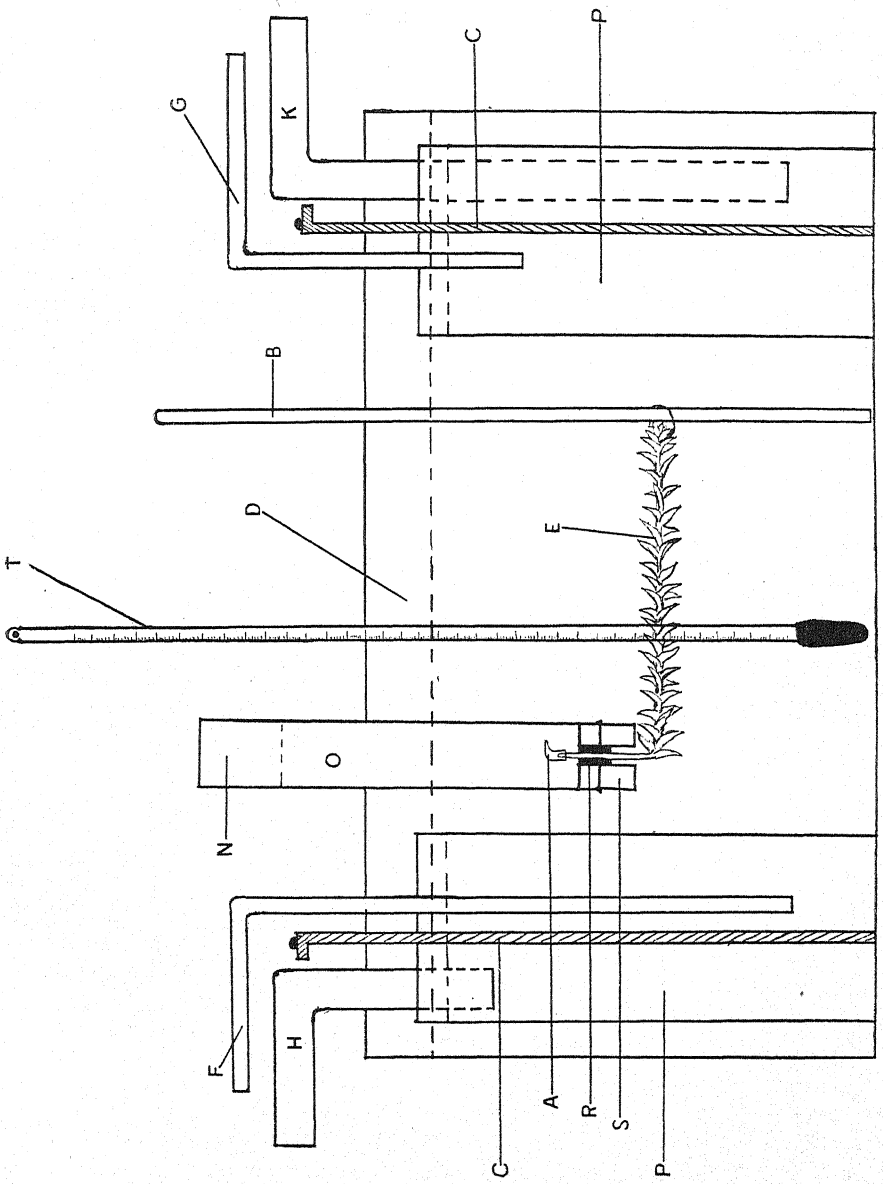
2. Methods Employed.

In spite of its inherent defects, it was decided to employ the bubbling method on account of its simplicity. The material of *Elodea canadensis* was obtained from Chelsea Physic Gardens, with the exception of that used in October, November, and December 1925 and January 1926, which came from the Botanic Gardens of Regent's Park. In the late autumn and winter, and occasionally in the summer, if the temperature rose very high, the *Elodea* was found to be in an unsatisfactory state, assimilating very irregularly and slowly. During these periods the material was taken from the Botanic Gardens as it was found to be in a better condition than that growing at Chelsea. It was usually necessary to bring fresh *Elodea* into the laboratory every fortnight.

Wilmot's (6) modification of the bubbling technique was employed. It was found essential that the 'glass shoe' (A in Figure) should fit closely over the shoots, and that the opening at the end of the capillary should be very small.

The *Elodea*, E in Figure, was placed in a rectangular glass vessel, D, 24 cm. long, 14 cm. wide, and 14·5 cm. deep.[1] In this vessel stood at each end a porous pot, P (12·5 cm. high and 4·7 cm. diameter); these pots held the metallic electrodes, C. Through the vessel tap-water was kept continually flowing, the level being kept constant at about 2 cm. from the top of the vessel by means of siphons, H and K. The temperature of this water (recorded by the thermometer, T) was controlled by passing it first through a coil of 'compo' tubing lying in a vessel of water heated by a small Bunsen burner. Through each porous pot a stream of tap-water passed, the level being kept constant by means of siphons F and G, the products of electrolysis appearing at the electrodes being thus carried away without coming in contact with the plant. Over the vessel was suspended a gas-filled lamp of 100 watts, placed centrally 13 cm. above the level of the *Elodea* shoot.

[1] From October 1925 to May 1926 a similar vessel, but about 1 cm. less in height, was used; allowance was made for this in calculating the current density.



For description see text.

The electrodes employed consisted usually of aluminium plates of a breadth about the diameter of the porous pots and of rather greater length; in experimental sets 1 and 5 thin platinum sheet was used. For currents between 150 and 250 milliamp. the voltage (220 D. C.) of the main was employed direct, since the resistance of the tap-water was considerable. For lower currents a resistance of 2,200 ohms was inserted in the circuit, and for still lower currents, 2–4 volts from accumulators or dry batteries were employed, using large resistances in the circuit, or smaller resistances as shunts. The currents passing were read on a Weston milliammeter or on a microammeter reading to 1·0 microamp. which could be suitably shunted for larger currents. In the experiments in which very small currents were employed some trouble was caused by thermo-electric effects due to the heating (by the lamp used for illumination) of the brass terminals attached to the aluminium electrodes, with the result that a small current was set up through the experimental vessel. This was avoided by replacing the brass terminals by soldered aluminium wires, or more simply by placing in the circuit a megohm resistance so that the current due to the thermo-electric effect became negligible. The current density was calculated in terms of the area of cross-section of the mass of water between the electrodes, which was about 1720 cm.^{2}. The living *Elodea* plant has a much higher resistance than that of the water, so that the current passing through it must be very small; its intensity is quite unknown.

3. Experimental Procedure.

The glass vessel was filled with water and the temperature regulated so that it remained constant to within 1° C. throughout the experiment. Different temperatures (20–27° C.) were used for the various experiments, since it was found that *Elodea* taken at different times of the year gave the maximum rate of bubbling at different temperatures.[1]

A piece of *Elodea* between 8 and 12 cm. long was carefully selected and cut off with a razor under water; if bubbling was regular a glass shoe of the Wilmot (6) type was fitted on to the cut end, which was then pushed gently through a hole in a rubber cork, S in diagram. The hole was then filled with a warmed wax mixture, R (vaseline eight parts, paraffin wax one part), which when cool made an air-tight joint and held the shoot firmly. A glass tube, N, containing distilled water, O, was fitted over this cork as described by Wilmot. The *Elodea* shoot was arranged as nearly as possible in the middle of the rectangular glass vessel and at a depth of about 6 cm. The glass tube containing the distilled water was held in position by a clamp.

[1] This was determined for samples of *Elodea* taken at different times of the year by placing material in beakers of water at different temperatures and noting the rate of bubbling under the illumination of a lamp.

The cut end of the shoot was tied very carefully with a piece of thread to a glass rod, B, so that it could be kept straight. When the plant was required to lie across the direction of the current it was placed in the centre of the vessel equidistant from the electrodes. The distance of the electrodes from the plant varied in the different experiments; if the current was to be high the electrodes were placed closer together. Before each experiment the distance between the electrodes and the exact position of the *Elodea* shoot in the water were noted. If the plant was to lie in the direction of the current it was so placed that the apex and base were the same distance from their respective electrodes. The lamp was suspended over the centre of the vessel before the beginning of each experiment.

The first reading was taken only after the *Elodea* had been in position for thirty minutes, for the bubbling rate at first decreases until there is a steady rate of diffusion of CO_2 to the shoot [(6), p. 310] though the procedure differed from that of Wilmot owing to the water round the *Elodea* being in constant slow movement. If during this half-hour period the *Elodea* shoot exhibited marked irregularities in the rate of bubbling, it was rejected and a new experiment set up.

During the experiment air bubbles collected on the surface of the leaves and stem of the shoot; these were removed at the beginning of the experiment, and afterwards every thirty minutes by the gentle use of a camel's hair brush; a period of ten minutes was allowed after such a removal before the next reading was taken. Readings were taken every five minutes, so that there were five readings spread over a period of twenty minutes, the bubbles were then removed and the sixth reading taken at the end of ten minutes. At each reading the time required for a fixed number of bubbles to be given off was recorded. This was repeated twice and the mean of the three readings taken, from which the number of bubbles given in the tables was calculated. The usual record was the time taken for five bubbles, but if bubbling was very active the time required for ten bubbles was employed.

Observations on each plant were made during three half-hourly periods, three counts being taken each five minutes except for the ten-minute brushing interval. For each half-hour period at least fifteen bubble counts were thus available for calculating the mean rate during that period. In the experimental sets the current was applied during the second half-hour and then cut off, the rate of bubbling during the third period being followed for any possible after-effect.

For each strength of current the three periods were studied in ten separate plants, so that the experimental result for any particular strength of current is based on not less than 450 measurements. For each experimental set there was a corresponding control set in which the rate of bubbling was followed during the three half-hour periods, though of course

no current was applied during the second period. As the rate of bubbling is not constant, even in the control plants, the effect (if any) of the current was determined *by the change from period to period in the bubbling rate of the experimental plants as compared with the change from period to period in the rate of the control plants.*

If the *Elodea* was active it was usually possible to carry out thirty experiments within three to four weeks, enabling one to study the effect of one particular strength of current in two experimental sets (ten experiments each) and to determine the rate in ten control plants. The experimental sets and their corresponding controls always had the shoot placed in the same position in relation to the electrodes. Controls and experimental sets were done alternately so as to reduce to a minimum the effect of the variation of material with season.

In view of the inevitable variability of material from plant to plant results of the kind obtained can only be satisfactorily studied in a statistical manner; the probable errors of the means, and of the differences between the means, have accordingly been calculated.

It was of great importance to ensure that under the conditions of the experiment light was not keeping the rate of assimilation at a low level. Accordingly the bubbling rate of *Elodea* was first determined under an illumination of a 100-watt lamp at a given distance; the intensity of the illumination was then doubled when the bubbling rate was found not to differ markedly from the previous rate. Similarly lack of CO_2 could not have kept the rate of assimilation down, because with a rise of temperature the bubbling rate increased.

In sets 1, 2, 3 and 4 (Table III) the falling off of the bubbling rate might be ascribed to injury caused by the products of electrolysis. This could only be the case if the water in the porous pots was circulating too slowly to remove these products. If this were so, it would be expected that in the experiments in which the *Elodea* was nearest to the electrodes the greatest decrease in the bubbling rate would occur. That the reverse is the case can, however, be seen by referring to Table III. In sets 3 and 4 the *Elodea* was lying parallel to the current with its ends an average distance of 3·5 cm. from the respective electrodes; in these experiments the decrease in the bubbling rate was considerably *less* than in set 2, where the *Elodea* shoot was lying across the direction of the current with the electrodes on an average 10 cm. apart. Any decrease in the bubbling rate cannot therefore be attributed to electrolytic products.

4. Experimental Results.

The results obtained with a single plant on the 23rd of April, 1927, are shown in full in Table I so as to exhibit the method of experimentation.

TABLE I.

Time.	Temp.	Current in (milliamp.).	Time for five Bubbles (sec.).	Mean No. of Bubbles per min.	
11.30 a.m.	27·0	none	17·5 16·8 17·0	17·54	
11.35 a.m.	,,	,,	17·8 17·5 17·5	17·04	
11.40 a.m.	,,	,,	17·8 18·0 18·5	16·58	Mean of Readings of 1st Period **16·39.**
11.45 a.m.	,,	,,	19·2 19·6 20·0	15·31	
11.50 a.m.[1]	,,	,,	19·5 20·7 20·0	14·95	
12.0 noon	,,	,,	18·0 17·5 17·8	16·89	
12.5 p.m.	,,	63	18·8 19·0 19·0	15·84	
12.10 p.m.	,,	60	19·8 20·0 20·2	15·00	
12.15 p.m.	,,	60	19·5 18·2 20·5	15·46	Mean of Readings of 2nd Period **15·24.**
12.20 p.m.[1]	,,	60	19·8 19·6 20·5	15·02	
12.30 p.m.	,,	60	20·6 20·0 19·8	14·90	
12.35 p.m.	,,	none	20·5 21·0 21·2	14·35	
12·40 p.m.	,,	,,	21·0 21·2 20·8	14·29	
12.45 p.m.	,,	,,	20·4 21·0 21·2	14·37	Mean of Readings of 3rd Period **14·47.**
12·50 p.m.[1]	,,	,,	21·5 21·8 21·8	13·82	
1.0 p.m.	,,	,,	19·3 19·3 19·3	15·54	

[1] Bubbles removed at these periods.

Table II shows in the first column the means (for ten experiments) of the number of bubbles per minute as calculated from the readings taken during the first periods of the experiments; similarly the second column shows the means of the second electrified periods of the experiments. From these figures the mean decrease and the mean percentage decrease of the second period over the first were found; these results are also given in Table II.

TABLE II.

Ten Elodea Shoots parallel to Current (55 to 63 milliamp.). Current Density 0·319 milliamp. per cm.² Bud towards Positive Electrode. April 22nd to 29th, 1926.

1st Period (no current) Bubbles per min.	2nd Period (current on) Bubbles per min.	Increase or Decrease of 2nd Period over 1st.	% Increase or Decrease of 2nd Period over 1st.
15·53	14·50	−1·03	−6·63
18·36	18·75	+0·39	+2·12
16·39	15·52	−0·87	−5·31
14·18	14·09	−0·09	−0·64
11·16	12·25	+1·09	+9·77
17·47	16·59	−0·88	−5·04
45·27	40·60	−4·67	−10·32
30·12	29·83	−0·29	−0·96
19·13	17·86	−1·27	−6·64
17·49	17·16	−0·33	−1·89

Mean Decrease of 2nd Period over first = $-0·80 \pm 0·33$.
Mean % Decrease of 2nd Period over first = $-2·55 \pm 1·21$.

When the results given in Table II were compared with the behaviour of the corresponding controls it was found that the latter showed a mean percentage decrease of $2·32 \pm 0·86$. The change of rate in the control and experimental sets is thus almost exactly the same, the current has thus had no effect on the assimilation rate.

The whole of the results for the different strengths of current are given in Table III.

5. Discussion of Results.

The results obtained do not confirm those of Koltonski. In no case was an acceleration of assimilation obtained by means of the electric current. Current densities of 1·118 milliamp. per cm.² and higher were found to reduce the rate of bubbling, while those below that level produced no significant effect. It is possible that still weaker currents might produce some effect, but it seems improbable. The smallest current density used in this work (6×10^{-7} milliamp. per cm.²) were, it is to be noted, very much smaller than those explored by Koltonski.

TABLE III.

Showing Effect of various Strengths of Current on the Bubbling Rate (Bubbles per min.) of Elodea.

A. Plant across direction of current. *B.* Plant parallel with current, apex towards *negative* electrode. *C.* Plant parallel with current, apex towards *positive* electrode.

The column headed E II − E I gives for the *experimental* plants the difference between the bubbling rate for the 2nd (electrified) period and the 1st (non-electrified) period. Similarly C II − C I gives for the *control* plants the difference between the bubbling rates for the 2nd and 1st periods.

The last column (E II − E I) − (C II − C I) gives the difference between the bubbling rate in the two previous columns.

Date.	No. of Experiment.	Current (milliamp.).	Current Density (milliamp. per cm.^{2}).	Position of Plant.	E II − E I.	C II − C I.	(E II − E I) − (C II − C I).
Oct. and Nov., 1925 . .	1	155 to 230	1·118	A	−13·44 ± 1·55	−1·31 ± 0·43	−12·13 ± 1·61
Feb. to April, 1926 . .	2	170 to 227	1·153	A	−12·22 ± 1·89	+1·18 ± 1·08	−13·40 ± 2·16
	3	,,	,,	B	−6·70 ± 1·83	+0·09 ± 0·66	−6·79 ± 1·94
	4	,,	,,	C	−8·36 ± 1·94		−8·45 ± 2·04
Dec., 1925 and Jan., 1926	5	55 to 60	0·334	A	−0·12 ± 1·06	−0·38 ± 1·16	+0·26 ± 1·57
April to June, 1926 . .	6	50 to 60	0·319	A	−2·58 ± 1·10	−1·88 ± 0·47	−0·70 ± 1·20
	7	,,	,,	B	−2·89 ± 0·50	−2·32 ± 0·86	−0·57 ± 1·00
	8	,,	,,	C	−2·52 ± 1·21		−0·20 ± 1·55
June and July, 1926 . .	9[1]	7 to 10	0·048	A	−2·70 ± 1·01	−2·03 ± 0·95	−0·67 ± 1·39
	10[1]	,,	,,	B	−1·36 ± 0·51	−0·76 ± 0·56	−0·59 ± 0·76
	11[1]	,,	,,	C	−2·28 ± 1·30		−1·52 ± 1·42
June, 1926.	12[1]	0·37 to 0·80	0·003	A	−1·51 ± 1·60	−2·03 ± 0·95[2]	+0·52 ± 1·86
	13[1]	,,	,,	B	+1·94 ± 1·19	−0·76 ± 0·56[3]	+2·70 ± 1·31
	14[1]	,,	,,	C	−1·92 ± 5·22		−1·16 ± 5·25
Mar. and April, 1927 . .	15	0·40	0·002	A	−0·78 ± 0·29	−0·67 ± 0·48	−0·11 ± 1·03
	16	,,	,,	B	−1·97 ± 0·59	−1·45 ± 0·66	−0·52 ± 0·88
	17	,,	,,	C	−0·51 ± 0·44		−0·94 ± 0·79
Dec., 1926 and Jan., 1927	18	0·1	6×10^{-4}	A	−3·02 ± 0·90	−0·92 ± 1·12	−2·10 ± 1·44
	19	,,	,,	B	−2·46 ± 1·09	−2·59 ± 0·46	+0·13 ± 1·18
	20	,,	,,	C	−2·93 ± 0·79		−0·34 ± 0·91
May to July, 1926 . . .	21	0·06 to 0·135	5×10^{-4}	A	−3·47 ± 1·27	−1·67 ± 0·51	−2·80 ± 1·38
	22	,,	,,	B	+0·04 ± 1·03	−0·93 ± 0·71	+0·97 ± 1·25
	23	,,	,,	C	−0·78 ± 0·97		+0·15 ± 1·20
Nov. and Dec., 1926 . .	24	0·0001	6×10^{-7}	A	−1·72 ± 0·74	−0·92 ± 1·12[4]	−0·80 ± 2·60
	25	,,	,,	B	−0·34 ± 0·69	−1·14 ± 1·90	+1·48 ± 2·02
	26	,,	,,	C	−3·33 ± 0·45		−2·19 ± 1·95

[1] Sets of 5 instead of 10.

[2] Same control as for set 9.

[3] Same control as for sets 10 and 11.

[4] Same control as for set 18.

6. Summary.

The effect of electric currents on the rate of assimilation of *Elodea canadensis* has been studied by passing currents through the water, means being taken to prevent products of electrolysis reaching the plant. Assimilation was studied by the bubbling method, using Wilmot's bubble technique.

Current densities varying from 1·15 to 6×10^{-7} milliamp. per cm.^{2} were employed.

In none of the experiments was the electric current found to increase the rate of assimilation. The effect of the current was either negligible or depressant. In sets 1, 2, 3 and 4 of Table III, a current density of about 1 milliamp. per cm.^{2} caused a decided decrease in the rate of assimilation.

This work was undertaken at the suggestion of Professor V. H. Blackman, whom I have to thank for much help and criticism.

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The Moisture Relations of Terrestrial Algae.

IV. Periodic Observations of Trentepohlia Aurea, Martius.

BY

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With fifteen Figures in the Text.

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Introduction.

THE following paper describes observations on *Trentepohlia aurea* extending over two years from November 1925 to November 1927, during which time the alga was collected and examined at approximately fortnightly intervals. The main objects of study were the manner in which the alga reacted to changes in its environment and the seasonal fluctuations in its growth. In addition, the reaction of the alga to plasmolysing solutions and to artificial conditions of drought were studied, and these were found to throw some light on the reactions to natural conditions. The results of these observations are given in the first part of the paper. The growth-investigations, which were followed only during the second year and consisted in a statistical study of the periodicity in size of cell and rate of growth, form the second part.

Part I. Direct Observations and Experiments.

A. *The Condition of the Alga in Nature.*

(1) *Habitat and General Characters of the Material.* The *Trentepohlia aurea* investigated grew near the waterfall at Virginia Water. In this position it was confined to the rocks, but further from the fall plants were observed on the red roof-tiles of a small hut and at the base of a beech-tree. All these localities were partially shaded and faced in directions from west to north except in the case of the tiles, which faced approximately east.

Three distinct growths from different spots were selected for observation. These will be referred to as *Trentepohlia A*, *B*, and *C*.

Trentepohlia A, which forms the main subject of investigation, occurred in a number of spots, but mainly on very hard silicate rocks. The samples investigated were collected fortnightly from an erect stone facing west of north, situated about ten yards from the fall and having no other sources of moisture than that supplied by the atmosphere. The material was typical of the species, as described by Heering (10, p. 122). It grows in a thin mat, yellow to orange in colour and not more than one millimetre in thickness. About half this thickness is composed of living filaments mostly growing erect. The under-surface of the mat is formed by a sparse soil mixed with dead creeping filaments. The individual threads are densely aggregated.

The cells of the living filaments are usually cylindrical, 6 to 14 μ broad and from 15 to 35 μ long. Although there were numerous creeping filaments, very few of these were composed of the round cells usually found in such filaments. The wall, which varies in thickness from 0·3 to 1·5 μ, is composed of two layers, which are described in detail by Brand (1, p. 201) and by West and Hood (15, p. 246). The outer layer, which forms a

continuous sheath without any marked constriction, has a characteristic flaky appearance, and in a few young apical cells striations have been seen on it (Fig. 1, A). The inner layer forms a continuous envelope round the cell-contents and presents a smooth appearance.

The septa, though thinner than the outer walls, are generally well defined. The wall between the apical and second cells sometimes contains a small pore which may be closed in the manner shown in Fig. 3, G. Fig. 3, H shows a peculiar condition in which a cross-wall with a pore has been formed just above the normal cross-wall. No pits, such as those observed by Brand (1, p. 223) in *Trentepohlia aurea, Mart. f. punctata,* have been seen. The apical cells taper to blunt tips and bear caps (1, p. 203) which vary in length (up to 14 μ) at different times of the year, and have been fully described by West and Hood (15, p. 247) and Brand (1, p. 203). Damp periods appear to favour their production. They result from the continued production of pectose by the growing apical cell, the whole mass having the appearance of a hat with horizontally striated markings. These 'caps' sometimes appear longer than they really are owing to the presence of a shrivelled apical cell beneath. The whole cap is finally pushed off to one side by the growth of the apical cell.

The material is freely branched (Fig. 2, J). Most of the primary filaments carry from two to four secondary branches from which, less frequently, tertiary branches arise. The branch usually joins its parent cell approximately at right angles and generally originates from its upper part. Fungal filaments are often seen entwined with the alga. These appear to be all of one kind, and no fructifications were seen.

The sporangia are arranged in four different ways: there are sessile lateral sporangia (Fig. 4, C), intercalary sporangia (Fig. 4, D), apical sporangia (Fig. 4, B), and lateral sporangia seated on one-celled stalks (Fig. 4, A). The majority are of the first kind. Intercalary sporangia are rare. Both types of lateral sporangia are approximately spherical when young but tend to assume a more flask-like appearance on ripening. They have very rugose walls. The apical sporangia are more elongated and their walls are smooth like those of ordinary cells. The intercalary sporangia differ from the ordinary cells only in the presence of a lateral swelling which later develops an aperture.

Trentepohlia B is found growing between two rocks in a very shaded place. It differs from *Trentepohlia A* in its greener colour and in the internal appearance of the cells.

Trentepohlia C is found on the stones used for completing the embankment facing west above the crest of the waterfall. These stones are of soft texture and composed mainly of calcium carbonate. They are too far away from the fall to be subject to the spray. The filaments of *C* are wider than those of *A*, the breadth being from 10 to 20 μ. They are not nearly so

densely aggregated as those of *A*, and have a characteristic appearance under the microscope owing to their mode of branching (Fig. 3, E and F). The sporangia are mostly lateral and fewer in number than those of *A*. In other respects *C* resembles *A*. The calcareous nature of the stone, rather than its nearness to the stream, may account for the difference, since

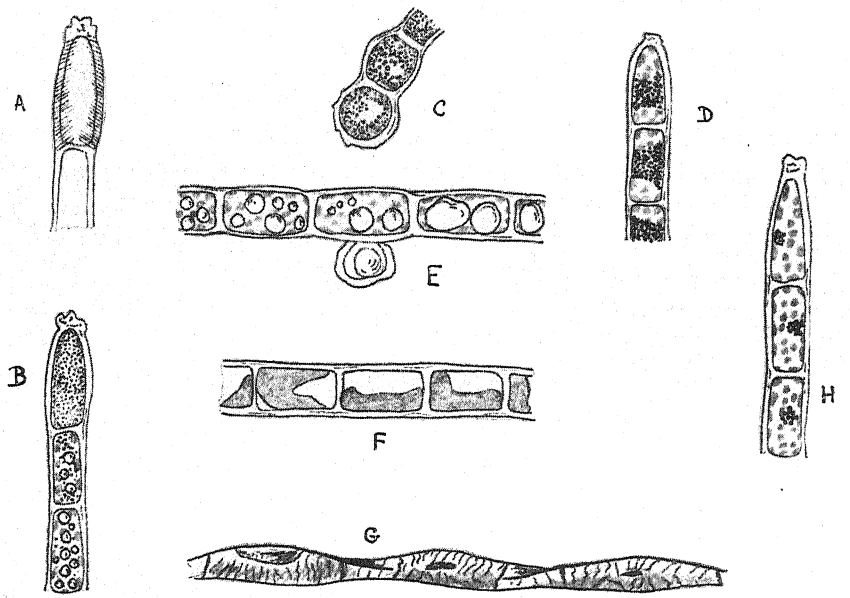


FIG. 1. A–G. *Trentepohlia A*. A, young apical cell showing striation. B and E, cells with oil globules. C, round cells showing vacuoles. D, condition of cells in a moist chamber. F, moribund cells. G, dry filament. H, *Trentepohlia B*, normal appearance. All Figs. × 490.

Trentepohlia A has been seen growing on an adjacent siliceous rock. Puymaly (13, p. 37) mentions that *Trentepohlia aurea* has a preference for calcareous soil. No transitional forms between *A* and *C* were observed.

(2) *Cell Contents.* The cells of *Trentepohlia A* are of two types differing in the condition of their contents (cf. Fig 1, B and E). Both contain globules of colourless oil which harbour the orange pigment (haematochrome), to which the alga owes its distinctive colouring. In the cells of the healthy type (Fig. 1, B, apical cell), to which the first ten cells of the filament usually belong, the globules are spherical, very small (diameter 1 μ or less) and very numerous, being dispersed throughout the cell. The disc-like chloroplasts are not visible, except during damp weather.

In the second type of cell (Fig. 1, B, 2nd and 3rd cells ; E), the globules are fewer and much larger (2–8 μ in diameter) and may be spherical or of irregular shape. Very often the chloroplasts are faintly seen. The basal cells, which are next to the dead substratum, are always of this kind. They appear to be moribund and the state of the globules may be an indication

of changes in the protoplasm preceding death. In material collected after a dry period many of the cells are of this second kind. Geitler (8, p. 77) has made the same observation. He states that in actively growing threads the globules become smaller till finally only minute ones are present, while during drought, when little or no growth takes place, the globules are large. Sporangia borne on the second type of cell generally retain their healthy appearance.

That cells with large globules are unhealthy is also shown by their more abundant occurrence after weeks of artificial desiccation. In material killed by heat or by the application of absolute alcohol all the cells show this appearance.

In *Trentepohlia B* the chloroplasts are distinctly visible. Only a few small globules of oil with haematochrome are present, generally clustered in a slightly eccentric position (Fig. 1, H). The cell contents of *C* are similar to those of *A*.

(3) *Differences in Wet and Dry Periods.* The subsequent observations refer to *Trentepohlia A* unless otherwise mentioned. On wet days the alga shows up much more clearly against its rocky background, and both it and its fine substratum of soil can be peeled off easily. On dry days it adheres very closely to the rock and has to be scraped off with a knife. The dry material absorbs moisture very rapidly, but dries much more slowly.

In damp weather the cells are turgid and often vacuolated, especially the round ones (Fig. 1, C). In the healthy cells the haematochrome globules are very frequently aggregated towards the centre (Fig. 1, D), leaving at either end an area of colourless protoplasm where the chloroplasts can be clearly seen. The same was observed by Gobi (9, p. 343) in material of *Trentepohlia umbrina* kept in a damp chamber. Whether there is a real reduction in the quantity of oil and haematochrome is doubtful, for if plasmolysed cells are allowed to recover in water the globules fill the cell as in the first type described above.

During several visits to Virginia Water the material was examined with a field microscope. After a drought the filaments, when mounted dry, had the shrivelled appearance shown in Fig. 1, G, which is similar to that observed after fresh material has been kept in a desiccator over calcium chloride for two or three days. No definite lines of shrinkage like those observed in the *Hormidium* stage of *Prasiola* by Fritsch (6, p. 10) were noticed, but successive cells had a tendency to flatten in planes at right angles to one another, so that the filaments had a twisted appearance. The haematochrome was collected towards one side of the cell. When such filaments were mounted in water, the cells were seen to be of the two kinds described in the last section.

To examine further the effects of drought on the cells some material was kept over calcium chloride in a desiccator for five weeks. The per-

centage of cells with large globules among the first ten cells of the filaments [1] in a count of 400 rose from 7 per cent. to 43 per cent. during this period. The rest of the cells had small globules.

(4) *Resistance to Desiccation.* *Trentepohlia A* will remain alive in a calcium chloride desiccator for as long as six months, retaining its normal

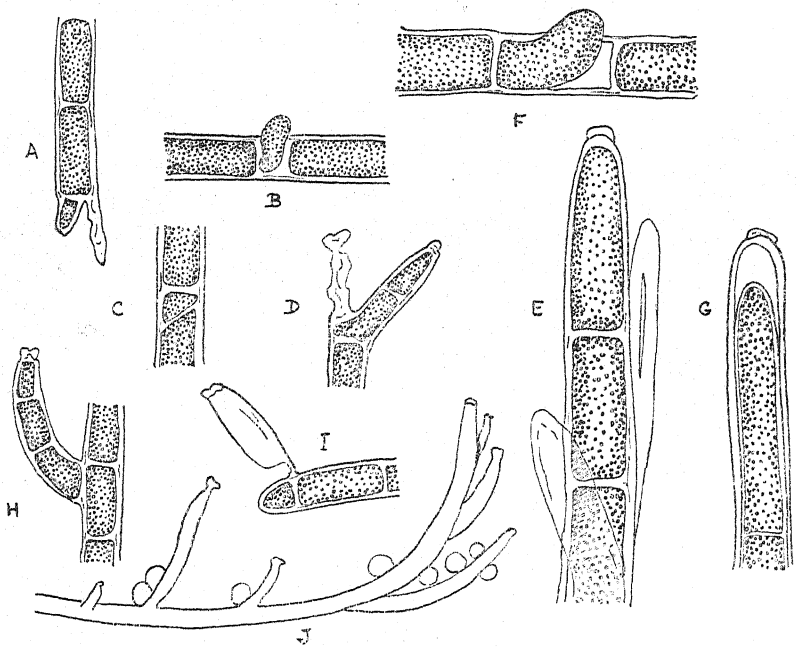


FIG. 2. A–D and H–J. *Trentepohlia A.* A–D, various modes of regeneration. H, J, branching. E–G, *Trentepohlia C*, proliferation. All Figs. × 490.

appearance. After this it turns green and finally grey, but the appearance of the green colour does not necessarily imply the death of all the cells. Some material, which had been in the desiccator for twenty-two weeks, had 79 per cent. of its cells (estimated on the first ten as usual) stained dark blue by toluidine blue.[2] This stain does not enter healthy cells, and in equivalent fresh material only 10 per cent. of the cells were very slightly stained. The cells of the desiccated material which failed to stain were of the type with many small globules. The protoplasm in the others was shrunken and neither globules nor chloroplasts were distinguishable, the whole of the cell contents being uniformly green (Fig. 1, F).

Trentepohlia A is able to regenerate in a number of different ways. Very often, if an intercalary cell dies, the one beneath cuts off a small triangular cell (Fig. 2, C) which grows out. Almost any small residue of

[1] The first ten cells of each filament were the only ones included in counts of plasmolysed cells.

[2] Eosin also stains the moribund cells.

protoplasm surmounted by a cell-wall can revive in a suitable environment and grow (Fig. 2, B, D).

Trentepohlia C usually exhibits a method of growth, styled proliferation by de Wildeman (16, p. 7), in which a cell, enclosed in the inner layer of the wall, pierces the outer layer and grows out as a branch. This has been seen to happen only after the dying away of the overlying cell. If

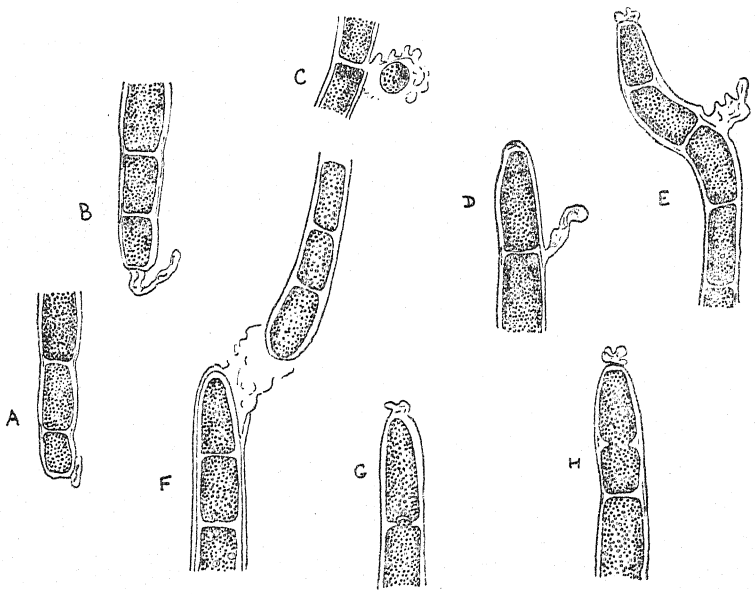


FIG. 3. A–H. *Trentepohlia A*. A, B, C, E, displacement of apical cap and apical cell. C, F, loosening of outer layers of cell-wall. G, H, pores in walls between apical cells. All Figs. × 490.

this is an apical cell, the latter is either pushed to one side or pierced by the growth of the second cell (Fig. 2, E, G). If an intercalary cell dies the cell below grows out to form a branch, which makes an acute angle with the main filament (Fig. 2, F).

(5) *Fragmentation.* The outer layer of the wall often disintegrates (cf. 1, p. 203), and the middle lamella between adjoining cells disorganizes, so that the cell-contents surrounded by the inner layer become free.

Dead tip-cells, after being pushed aside by the growth of a new tip-cell (Fig. 3, D, E), fall off by the disorganization of the outer layer. The pectose caps are shed in the same way (Fig. 3, A). Sometimes a whole branch, comprising up to five cells, is separated from the main one by this kind of fragmentation (Fig. 3, F). Fragmentation appears to take place more frequently in damp weather. No evidence of wind dispersal of these detached branches has been obtained.

(6) *Reproduction.* Zoospores were seen freely issuing from lateral and apical sporangia of *Trentepohlia A* on June 8th and 20th, 1926, and

July 7th and 20th, 1927, but at no other time during the two years. The sporangia were always attached to their filaments. In *Trentepohlia umbrina* Caspary observed swarming in May and June (2), Wille in October (17, p. 426), Deckenbach (3, p. 33) in February, and Brand (1, p. 216) in a mild winter. Brand (1, p. 216) suggests that swarming depends less on the calendar than on temperature and moisture, and this finds some support in my own observations. The temperatures in April and May of 1926 and 1927 were very similar, but the rainfall in 1926 is greater by 1·61 in. than in 1927. The actual figures are:

	1926.		1927.	
	Rainfall.	Average Temperature.	Rainfall.	Average Temperature.
	inches.		inches.	
April	3·04	50 F.	1·83	49 F.
May	1·63	54 F.	1·23	54 F.

This suggests that, given the right temperature, lack of moisture may be a limiting factor in the maturation of the sporangia.

The development takes place very slowly, and it was not until May of 1927 that zoospores were formed. At first they filled the cells, but just before ripening they appeared as a small spherical group (Fig. 4, H) radially arranged in the centre of the cell. This arrangement finally disappears and the scattered zoospores again fill the cell (Fig. 4, I). The apertures of the sporangia were so narrow that the zoospores had to squeeze their way out (Fig. 4, C). If they failed to do so during their period of activity they were left behind. Some of the zoospores of the year 1926 developed into filaments of two or three cells, without having left the sporangia (Fig. 4, J).

Immediately after liberation the zoospores were elongated and of irregular shape, probably due to their squeezing through the narrow opening (Fig. 4, F). Active movement lasted for about a quarter of an hour, and during this time the zoospores were oval (about 5–8·4 μ long, 2–5 μ broad, with cilia 17–18 μ long).[1] They finally rounded off and settled down (Fig. 4, E). Hildebrand (11, p. 84) mentions having seen zoospores of *Trentepohlia aurea var. tomentosa* in movement for hours at a time. In view of the fact that the swarmers from some sporangia of *Trentepohlia* have been proved to be of the nature of gametes (12, p. 328), it may be stated that no evidence of sexuality has been obtained in the zoospores here recorded.

As evidence of the vitality it may be mentioned that some material, in which zoospore formation was taking place abundantly, was left in 10 per cent. cane-sugar solution, which then dried up and remained in this con-

[1] The zoospores of *T. aurea var. tomentosa* recorded by Hildebrand measure 2–4 μ broad, 10–12 μ long, with cilia 20 μ long.

dition for two days. On transference to fresh water the zoospores were rapidly liberated.

(7) *Dispersal.* On more than one occasion, after rain, a part of the rock which had appeared to be uncolonized became yellow. Such material proved to be composed of numerous filaments consisting mainly of dead

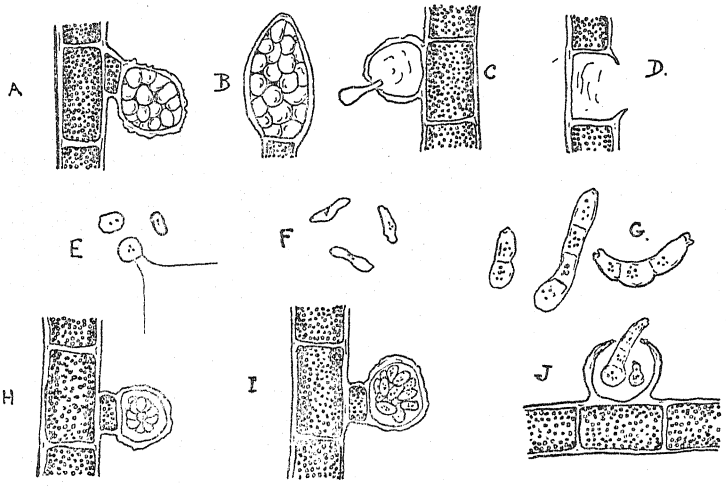


FIG. 4. A–J. *Trentepohlia A.* A–I, stalked lateral sporangia. B, apical sporangium. C and J, sessile lateral sporangia. D, intercalary sporangia. E, F, zoospores. G, young filaments. J, zoospores germinating within sporangia. H and I show arrangement of zoospores. C, the escape of a zoospore. All Figs. × 490.

cells, but with scattered living cells amongst them. Very often these new growths became indistinguishable in dry weather, and did not always reappear in subsequent wet periods.

In spite of various attempts to investigate the mode of dispersal of the alga it has not been certainly established. Marks were painted on the rock at the edge of existing growth in several places to see if there were indications of lateral spread, but no positive results were obtained. Several coverslips coated with egg-albumen were fixed by means of vaseline, to bare parts of a rock facing west to north-west, in the early part of the year. On examination of these coverslips after a month, only one bore a solitary zoospore: the others showed no signs of *Trentepohlia.*

It seems probable that zoospores, washed down the face of the rock in rainy periods, get lodged in small niches and germinate there. Young mosses, which also colonize the rock, had numerous young filaments of *Trentepohlia A* growing along the midrib of their partly folded leaves. The possibility of wind dispersal of fragments produced as described above must also be considered, but no definite evidence of its occurrence was obtained.

B. *Moisture Relations.*

(1) *Loss of Moisture during Drying.* The absorption of moisture is very rapid. It is absorbed by the cells and held between the filaments by capillarity. The moisture relations were first examined experimentally,

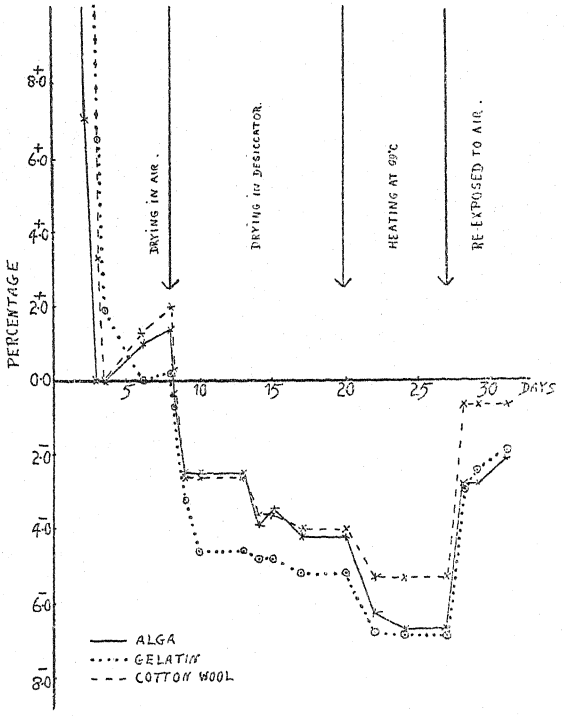


FIG. 5. Graphs showing decreasing water-contents of *Trentepohlia*, gelatin and cotton-wool during drying. The arrows mark the respective periods of drying in air, &c.

after the method by Fritsch (6, p. 4), a comparison being made with the behaviour of 10 per cent. gelatin and of cotton-wool. The alga and cotton-wool were soaked in water and allowed to drain for three hours in a Gooch crucible. These, as well as the 10 per cent. gelatin, were then placed in watch-glasses and allowed to dry in the air for eight days until their weights were practically constant. The materials were afterwards dried in a desiccator over concentrated sulphuric acid and, when the weights were again constant, heated at approximately 99° C. in a drying-oven, re-weighed, and allowed to stand in the air to reabsorb moisture. A watch-glass, used as a control, did not vary appreciably in weight.

Fig. 5 shows the water-content of the materials at various times during the experiment, expressed as percentage of the air-dry weight. The amounts of moisture lost during the heating, diminished by the amount reabsorbed

when the material was replaced in air, expressed as percentage of the air-dry weight were alga 2·1 per cent., cotton-wool 0·7 per cent., and gelatin 1·95 per cent. In the case of the alga this may be said to represent the amount of water held by the living machinery of the protoplast. The three graphs in Fig. 5 are very alike. The correspondence between the alga and cotton-wool is in places remarkable. This suggests that the absorption of water by *Trentepohlia* is by imbibition and is in the main a physical process, and that the large bulk of the water is held by the walls. It may be noted, however, that in the parts of the graph corresponding to the heating there is less correspondence between alga and cotton-wool and more between the former and the gelatin, which perhaps means that the moisture lost during heating is that absorbed by the colloidal protoplast.

(2) *Plasmolysis Experiments.* Plasmolysis, as a method of investigating the condition of the cell-contents, has been used extensively by Fritsch and Haines (7). It has been applied to *Trentepohlia* to test its reactions both to natural and to artificial conditions. The cells were frequently so resistant to plasmolysing solutions of ordinary strength that it was necessary to use 25 per cent. Tidman's sea-salt solution.

The two types of cell described above react differently to plasmolysing solutions. When a healthy cell is distinctly plasmolysed the oil and haematochrome lose their definite segregation into small globules. The latter seem to run together and produce a more viscous mass. When the second type of cell is plasmolysed, the protoplasm contracts without any alteration in the large globules. This type of cell is more irregular in its reactions to plasmolysis; cells of the same filament sometimes plasmolysing very strongly and sometimes not at all. Since very few cells of the second type occurred among the apical ten cells of the filaments, it was decided to restrict the observations to these.

The following method was adopted. The material was first cleaned to remove soil particles which might become dissolved in the plasmolysing solution. The threads were then mounted in the latter on a slide covered with a watch-glass and left for half an hour, a period of time which had been found to be sufficient for the solution to have its full effect. While these threads were being examined a fresh lot of material was treated with the solution and used as soon as ready. This frequent use of fresh material prevented errors that might have arisen through concentration of the plasmolysing solution.

The cells were classified simply as 'not noticeably plasmolysed' or 'noticeably plasmolysed'. The experiments fall into two groups—the special tests of 1926 and the periodic tests of 1927. In each of the special tests the effect of the plasmolysing solution on about 500 cells was observed. After these tests had been carried out an experiment was made to test the reliability of the method. The reactions of 3,000 cells to the salt solution

were recorded. The percentage of plasmolysed cells in six successive groups of 500 were 22·2, 18·1, 18·9, 22·6, 17·6, and 21·4. The mean of these figures is 19·7, so that the errors in the separate results vary from 6·0 to 12·4 per cent. Such errors would not affect the main conclusions reached.

It will be seen, however, that the percentages for the three successive thousands are 20·2, 20·8, 19·5, the greatest variation from the mean being 4 per cent. As a result it was decided to treat 1,000 cells in each of the periodic tests.

(*a*) *Special Tests.* Material of *Trentepohlia A* was kept for a long period in a desiccator over pure sulphuric acid and samples of it tested with salt solution at intervals. The results are shown in Table I.

TABLE I.

Successive Estimations of Plasmolysis (with 25 per cent. Sea-salt) of Material of Trentepohlia A *exposed to Drought over Pure Sulphuric Acid.*

Duration of Desiccation.	Number of Cells Treated.	Noticeably Plasmolysed.
		%
0 days	627	83·0
2 „	551	80·6
4 „	595	99·7
8 „	613	51·9
7 weeks	412	24·8

The curious results obtained on the fourth day may be compared with those obtained by Fritsch and Haines (7, p. 696) in their work on Moss protonema, where on the fourth day and afterwards the percentage of unaffected cells decreases and of slightly plasmolysed cells increases.

Trentepohlia B was also kept in a desiccator over concentrated sulphuric acid for eight days. Its reactions to plasmolysing solutions are shown in Table II.

TABLE II.

Successive Estimations of Plasmolysis (with 25 per cent. Sea-salt) of Material of Trentepohlia B *exposed to Drought over Pure Sulphuric Acid.*

Duration of Desiccation.	Number of Cells Treated.	Noticeably Plasmolysed.
		%
0 days	483	99·0
2 „	422	91·0
4 „	356	92·5
8 „	187	78·0

The material was very unhealthy on the eighth day. There was again a slight drop in the percentage of unaffected cells on the fourth day, but it was not as noticeable as in the case of *Trentepohlia A*. These experiments

suggest that, in the absence of oil and haematochrome, the protoplasm does not so readily lose its power of plasmolysing on exposure to drought, but the results may be conditioned by other less obvious differences between *A* and *B*.

Similar tests were made on material kept in a damp chamber, the results for the two forms being shown in Table III.

TABLE III.

Successive Estimations of Plasmolysis (with 25 per cent. Sea-salt) of Material of Trentepohlia A *and* B *kept in a Damp Chamber.*

Duration of Exposure to Moisture.	*Trentepohlia A.*		*Trentepohlia B.*	
	Number of Cells Treated.	Noticeably Plasmolysed.	Number of Cells Treated.	Noticeably Plasmolysed.
		%		%
0 days	627	83·0	483	99·0
1 ,,	542	94·6	471	100·0
3 ,,	652	95·1	513	99·2
7 ,,	356	97·7	350	100·0

TABLE IV.

Estimations of Plasmolysis and Counts of Dead Apical Cells of Trentepohlia A, *and Meteorological Data for the Periods preceding Collection.*

Date.	Percentage of Cells Noticeably Plasmolysed.	Percentage of Dead Apical Cells.	Average Temp. F. Preceding 5 days.	Total Rainfall for Preceding 5 days.	Percentage Humidity on Day of Visit.	
				(mm.)	9 a.m.	9 p.m.
Nov. 15	26·3	0·0	47	30·9	80	96
,, 27	40·9	6·0	40	5·1	100	98
Dec. 12	28·9	8·2	42	0·6	100	100
,, 29	4·0	8·0	35	2·0	57	87
Jan. 16	23·4	5·3	42	8·6	93	95
,, 31	33·7	6·7	44	16·2	83	81
Feb. 13	7·8	13·6	31	0·1	97	98
Mar. 6	4·9	24·0	46	10·6	79	68
,, 20	2·7	21·6	46	0·2	65	92
Apr. 4	3·0	10·5	43	9·4	85	94
,, 24	2·7	8·0	52	0·0	67	78
May 8	0·0	18·0	61	1·4	64	65
,, 23	0·5	14·9	52	7·9	54	66
June 9	2·9	19·7	52	12·1	65	68
,, 23	1·0	11·2	57	11·5	99	83
July 7	5·6	14·7	61	4·3	71	87
,, 26	2·7	0·0	61	0·5	84	94
Aug. 19	39·2	2·3	58	30·7	89	82
Sept. 5	4·5	11·7	62	0·0	87	83
,, 21	24·4	3·9	56	13·0	87	94
Oct. 8	52·2	11·4	48	0·1	100	93

The various experiments show that in *Trentepohlia*, as in other terrestrial algae (7), loss of the power to plasmolyse is one of the usual responses to drought.

(*b*) *Periodic Tests.* In 1927, fortnightly estimations of plasmolysis in 1,000 cells of *Trentepohlia A* were undertaken. The percentage of cells noticeably plasmolysed is shown in Table IV, which gives also the total rainfall and the average daily temperature during the five days preceding collection, as well as the percentage humidity on the day of collection. The results are shown graphically in Fig. 6 A.

This indicates a seasonal tendency for the cells to plasmolyse more easily in the early and late periods of the year. Superposed on this seasonal fluctuation are irregular variations which show a correlation between plasmolysis and moisture conditions, the tendency being for a higher percentage of cells to plasmolyse after a wet period. For example, on November 15th, 1926, the percentage of cells noticeably plasmolysed was 26·3 per cent. 30·9 mm. of rain fell during the previous five days, and on the day of collection the humidity was very high. On November 27th, 40·9 per cent. of the cells were noticeably plasmolysed. The rainfall for the previous five days was only 5·1 mm., but the humidity on the day of visit was high. By contrast, on December 29th, after five days with a total rainfall of only 2·0 mm., the humidity was rather low and only 4 per cent. of the cells were noticeably plasmolysed. A month later (January 31st, 1927), after five days in which 16·2 mm. of rain fell, 33·7 per cent. of the cells were plasmolysed.

Turning to the summer, it is clear that in May and June the cells are very resistant to plasmolysis. But the percentages for May 8th, May 23rd, June 9th, June 26th, namely, 0·0 per cent., 0·5 per cent., 2·9 per cent., 1·0 per cent., show a clear correlation with the rainfall preceding these four dates, which was 1·4, 7·9, 12·1, 11·5 mm., respectively. In July and August the cells tend to plasmolyse more easily, but the correlation with the rainfall is again clear.

Owing to the seasonal fluctuation, this correlation is not easily seen when the results for widely separated dates are compared. Sometimes it is not noticeable even when the dates are consecutive. For example, on February 13th, after a rainfall of only 0·1 mm., the percentage of plasmolysed cells was 7·8, while on March 6th, after 10·6 mm. of rain, it was 4·9. Two factors seem to have been at work to produce this result. In the first place the comparatively low resistance of the cells may be related to the high rainfall (30·9 mm.) in the first week of February, aided by the high humidity of the five succeeding days. At the same time the seasonal fluctuation is tending to increase the resistance of the cells to plasmolysis.

A similar result occurred on October 8th, 1927, when the percentage of plasmolysed cells was 52·2, the highest for the year. Only 0·1 mm. of

rain had fallen during the previous five days, but 20·8 mm. fell in the five days before that, whilst the average humidity for the five days preceding collection was 94 per cent. of saturation. In addition, it is to be remembered that the cells are, on the average, much more easily affected at this time of the year.

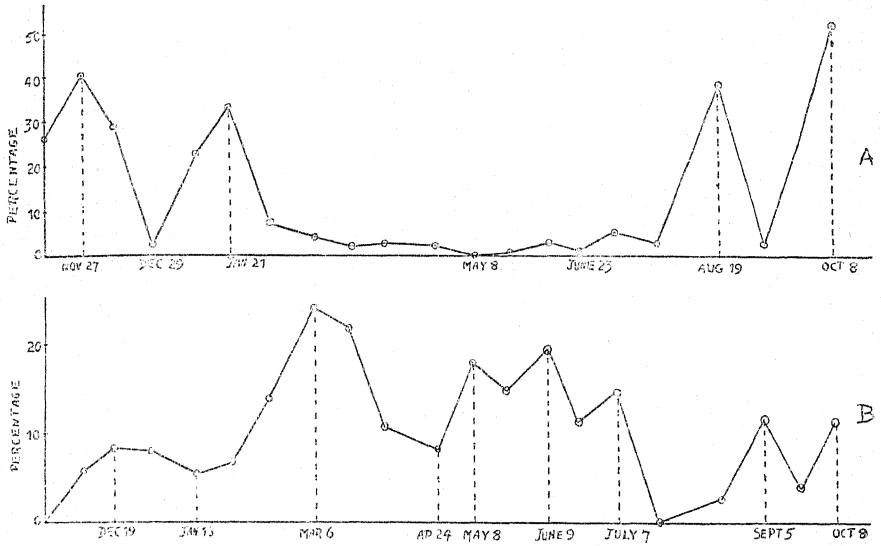


FIG. 6. *Trentepohlia.* The upper graph A shows percentage of cells plasmolysed, the lower B the percentage of dead apical cells at different periods of the year.

(3) *Occurrence of Dead Apical Cells.* The percentage of dead apical cells among the 1,000 cells estimated (approximately 100 filaments) are given in Table IV. The results are shown graphically in Fig. 6 B. It is clear that there is a tendency for greater mortality of the apical cells in early spring and summer, especially the former. This corresponds to the period of low humidity. No direct correlation between the percentage of dead apical cells and rainfall or temperature data could be found, though this is possibly due to insufficient cells being counted. There is, however, a relation to the plasmolysis graph, that for dead apical cells rising when the latter falls and vice versa.

PART II. STATISTICAL INVESTIGATION OF GROWTH.

(1) *Method.* During the period November 1926 to November 1927 a statistical investigation of the growth of the cells of *Trentepohlia A* and its relation to the environment was undertaken. Measurements were made at approximately fortnightly intervals of:

(i) The number of cells contained in a definite length of filament (140 μ) measured from the apex backward.

(ii) The length of the second cell.

(iii) The length and breadth of the apical cell.
(iv) The length and breadth of the sessile lateral sporangia.

The material for these investigations was invariably taken from the same area of four square feet of rock surface, so as to ensure uniformity. For the measurements mentioned under (i) a length corresponding to 50 units (140 μ) of a measuring eye-piece was adopted. Parts of a cell less than one-quarter of a unit were ignored, while parts greater than one-quarter and less than three-quarters were counted as one-half. The length of the second cell was measured from middle lamella to middle lamella, that of the apical cell from the outer margin of the extreme tip to the middle lamella between it and the second cell. The caps were not included. The breadths of the cells were measured to include both lateral walls. Only the cavities of the sporangia were taken into consideration, at their greatest breadth and length, since the walls were so rugose that they would have contributed an inconstant error to the measurements.

The measurements at once revealed that cells of the same class vary considerably amongst themselves in dimensions. For example, apical cells varying in length from 2 to 20 units (5·6–56 μ) have been found. Such a degree of variation necessitates the measurement of large numbers, if the results are to be trustworthy and a statistical treatment is essential. 400 measurements were made in the case of (ii), (iii), and (iv), and 200 in the case of (i).

(2) *Frequency Distributions.* The method here adopted (Fisher, 5, p. 37 et seq.) is to group the measurements, plotting the number in each group against the mean size of the group to give a frequency curve. Curves drawn from a large number of measurements, in which the causes of variation are not systematic, are usually of the symmetrical (normal) type (5, p. 45). In an unsymmetrical (skew) curve the highest point (mode), representing the magnitude which occurs most frequently, will not generally coincide with the mean.

If the sample is homogeneous and a sufficient count has been made the curve should be smooth. If, in addition, it is symmetrical and has only one mode the mean is significant. If σ is the standard deviation, $\frac{\sigma}{\sqrt{n}}$ is called the standard error. A variation in mean from one fortnight to another if more than twice the standard error is evidence of a real variation in the material. Smaller changes may be due to accidents of sampling. With $n = 400$ this gives 1/10 of σ as the least significant change.

When the curve departs from the normal, but has a single well-defined maximum, the mode may be more significant than the mean. An example of this is clearly shown in the measurements of the sporangia.

(3) *Meteorological Data.* The data were obtained from the Meteoro-

logical Station at Heatherwood Hospital, Ascot, that being the nearest station to Virginia Water. They are shown graphically in Fig. 7.

(4) *Average Size of Cells.* The number of cells counted from the apex backwards in a total length of 50 units of the micrometer scale (140 μ) varied from 4 to 11 (average length of cell, 35–12·7 μ). The frequency curves of all the fortnightly samples were approximately normal in type. As an example, we consider the 400 measurements of March 6th, 1927.

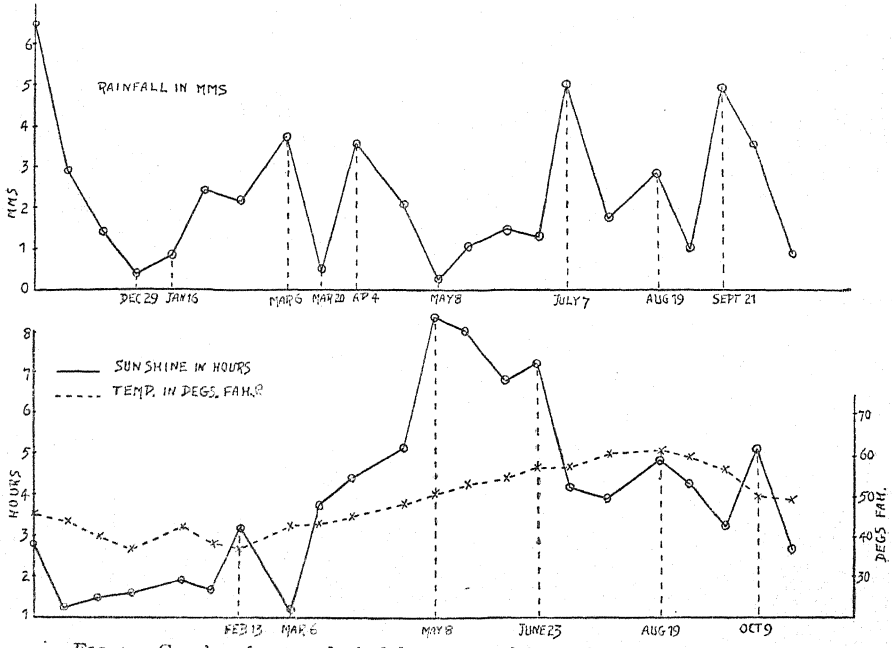


FIG. 7. Graphs of meteorological data averaged for 14 days preceding collection.

A psychological tendency to place rather more at the integer values and less at the halves was noticed. To eliminate this error the measurements were regrouped equally at the integers. For example, of the twenty-four at $6\frac{1}{2}$, twelve were placed at 7, the rest at 6. The results for March 6th, 1927, are then as follows:

No. of cells in 50 units [1]	5	6	7	8	9
Percentage Values	8·5	30·5	43·75	14·0	3·25

When these figures are plotted they give the curve shown in Fig. 8, which is almost normal. The curve for April 4th is also shown. Similar frequency curves occurred on most occasions, the standard error never exceeding 0·08. Therefore it may be concluded that sufficient cells were measured; that the material is homogeneous; and that the mean is significant as a measure of the average size of cell.

[1] One unit = 2·8 μ

A variation of more than 0·15 in the mean is significant and represents a real change in the material.

The means and standard errors are shown in the first column of Table V (p. 191), and the means are shown graphically in Fig. 9. The variations in the means cover a range of 1·6, or twenty times the greatest standard error. The standard deviations vary only by 0·24, a change which is hardly significant.

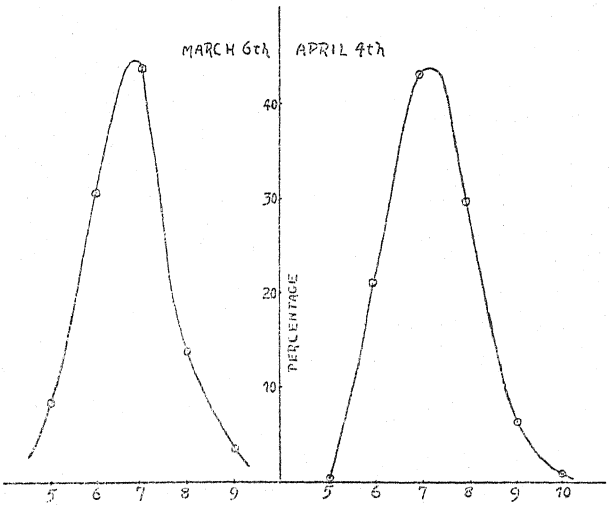


FIG. 8. *Trentepohlia.* Frequency curves for number of cells occurring in 50 units (140 μ).

In its extremest variation the mean is related to the rainfall, but there is also a temperature effect. The lowest mean (6·5), that is the longest average cell (21·5 μ), occurred on December 29th, after an exceptionally cold and dry period. The average daily rainfall for the fortnight preceding this was 0·32 mm., which, with one exception, was the lowest of the year. The average temperature was 37° F., the lowest recorded for any fortnight during the year.

The highest mean (8·1) occurred on July 7th, after a fortnight in which the average daily rainfall was 5·1 mm., the highest of the year. Exceptionally high means (7·7–8·0) occurred also on September 21st, October 8th, and October 25th, after the heavy rains of September and early October. If growth in length is practically confined to the apical cell (see below), these results imply that, during periods of dry weather, and especially if the temperature is low, the apical cells divide rarely, so that very long segments are cut off. Alternatively, in periods of wet weather, division of the apical cell must occur before the latter has reached nearly so great a length, so that the segments are shorter and a larger number fall into the length of filament measured.

A comparison with the rainfall for the five days before each of these

dates fails to show a similar correspondence. This indicates that a period of a fortnight or more is required for the lowest (i. e. 6th or 7th) cell to be affected by the prevailing meteorological conditions. It also implies that some six or seven divisions of the apical cell occupy about a fortnight. In fact the high mean (8·0) of October 25th appears to be related to the high rains of more than a fortnight previously.

An influence of temperature is recognizable on March 20th and May 8th. The average daily rainfall for the fortnight preceding March 20th was only 0·5, but the mean number of cells was 7·4. This is larger than on the dates before and after March 20th, and much larger than that for December 29th. This suggests that the moderately high temperature in March is tending to accelerate cell-division and reduce the size of the cells. This is confirmed by the results for May 8th, when despite low rainfall (mean 0·2 mm.) the cells are only of moderate size, rather smaller in fact than in the previous period of higher rainfall. The fortnight preceding May 8th was a period with abundant sunshine and high day temperature.

To sum up, high rainfall and high temperature both favour a small average cell, suggesting rapid cell division. The largest cells appeared after a dry cold period, the smallest after a wet, relatively warm period.

TABLE V.

Means, with Standard Errors, of Number of Cells in 50 units (140 μ), and of Length of Second Cell, and Average Widths of Apical Cells at the Dates of Collection.

Date.	No. of Cells in 50 units. Mean.	Length of Second Cell.[1] Mean.	Apical Cell.[1] Mean Width.
Nov. 15, 1926	7·1 ± 0·07	7·1 ± 0·07	3·3 ± 0·04
„ 27, „	7·4 ± 0·07	7·0 ± 0·06	3·3 ± 0·03
Dec. 12, „	7·3 ± 0·07	6·9 ± 0·06	3·4 ± 0·03
„ 29, „	6·5 ± 0·07	8·3 ± 0·06	3·8 ± 0·04
Jan. 16, 1927	7·5 ± 0·07	7·3 ± 0·07	4·0 ± 0·04
„ 31, „	7·2 ± 0·05	7·4 ± 0·06	4·1 ± 0·04
Feb. 13, „	7·0 ± 0·06	—	—
Mar. 6, „	6·7 ± 0·06	7·2 ± 0·05	4·2 ± 0·04
„ 20, „	7·4 ± 0·07	6·9 ± 0·06	3·9 ± 0·04
Apr. 4, „	7·2 ± 0·07	7·0 ± 0·06	3·7 ± 0·05
„ 24, „	7·0 ± 0·07	7·3 ± 0·07	4·0 ± 0·04
May 8, „	7·2 ± 0·08	7·4 ± 0·07	4·0 ± 0·04
„ 23, „	7·2 ± 0·07	7·3 ± 0·07	4·1 ± 0·05
June 9, „	6·9 ± 0·07	7·3 ± 0·07	3·7 ± 0·05
„ 23, „	7·1 ± 0·08	7·0 ± 0·06	3·8 ± 0·04
July 7, „	8·1 ± 0·07	6·3 ± 0·06	3·4 ± 0·04
„ 26, „	7·7 ± 0·08	6·9 ± 0·07	3·4 ± 0·03
Aug. 19, „	7·8 ± 0·07	6·5 ± 0·06	3·1 ± 0·03
Sept. 5, „	7·1 ± 0·07	6·4 ± 0·06	3·1 ± 0·03
„ 21, „	7·7 ± 0·07	6·0 ± 0·05	3·1 ± 0·03
Oct. 8, „	7·9 ± 0·07	7·2 ± 0·07	3·4 ± 0·04
„ 25, „	8·0 ± 0·07	7·2 ± 0·07	3·8 ± 0·04

[1] The lengths of the cells are given in units ; one unit = 2·8 μ.

(5) *Length of Second Cell.* The lengths of the second cell were distributed symmetrically about their mean, the frequency curve being nearly normal in type. For example, in the 400 cells measured on April 4th the

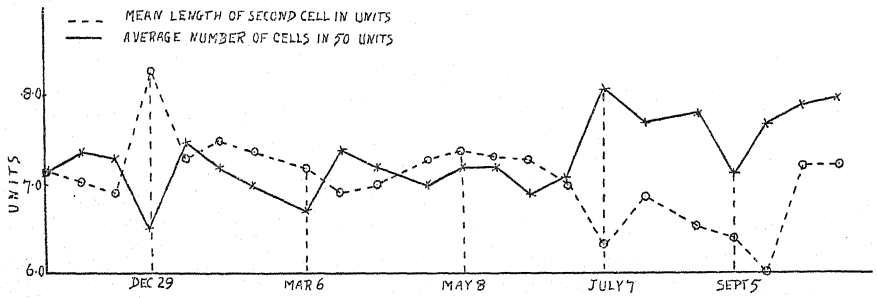


FIG. 9. *Trentepohlia.* Graphs of means of number of cells in 140 μ and of lengths of second cell (in units).

percentages having lengths from 3–12 units of the scale (8·4–33·6 μ) were as follows:

Length (units)	3	4	5	6	7	8	9	10	11	12
Percentage	0·1	2·1	8·2	22·0	35·7	20·6	8·2	2·4	0·6	0·1

These figures were obtained by regrouping the measurements in the same way as before. The frequency curve based on them is shown in Fig. 10. A less normal but still symmetrical curve is that for December 29th shown in the same figure and drawn from the following data:

Lengths	4	5	6	7	8	9	10	11	12	13
Percentage	0·1	0·6	7·5	24·5	29·6	28·8	7·3	1·1	0·4	0·1

This represents about the greatest departure from the normal. It may therefore be concluded that the material is homogeneous, the sample sufficiently large, and the mean a significant measure. The means and standard errors are shown in Table V. The greatest standard error is 0·07, so that changes in the mean of as much as 0·2 are significant and not due to accidents of sampling.

The variations in the mean, plotted in Fig. 9, confirm the conclusions of section (4). The largest cells (mean 8·3 units = 23·2 μ) occurred on December 29th, and the smallest (mean 6·3 units = 17·6 μ) on July 7th. On May 8th the effects of low rainfall and high temperature counteract one another and produce a cell of only moderate size (7·4 units = 20·7 μ). The steady decline in the length of the second cell from July 26th to September 21st, a period of heavy rain and moderate temperature, is also noticeable. An interesting feature is to be observed on October 8th, when the second cell shows a marked increase in size (6·0–7·2 units = 16·8–20·2 μ) over that on September 21st, although the number of cells in 50 units has slightly increased. Although the average daily rainfall from September 21st

to October 8th was high (3·6 mm.), the total rainfall for the five days preceding October 8th was only 0·1 mm., and it is possible that the change to dry weather has affected the second cell but not those further from the apex.

(6) *Length of Apical Cell.* The lengths of the apical cells gave very different results from those just considered. The frequency curves showed

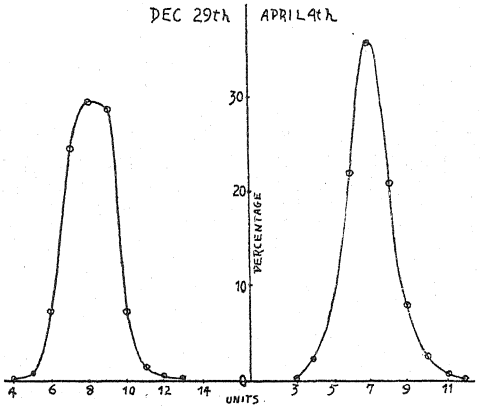


FIG. 10. *Trentepohlia.* Frequency curves for lengths of second cell.

a variety of forms, usually with more than one peak, the normal type very rarely occurring. Four of these curves are shown in Fig. 11. The irregularity of these curves cannot be due to errors of measurement. Errors greater than half a unit were very unlikely to occur, and so no measurement would fall into the wrong group. It is possible, but not likely, that the curves would be regular if more cells were measured. This could not have been done without abandoning some of the other work, since it was essential that all the measurements should be made while the material was fresh.

Assuming that the shapes of the curves give a true representation of the states of the material at the times of collection, their irregularities must be related in some way to the growth of the apical cells. West and Hood (15, p. 242) state that growth is confined to the apical cell, and Brand (1, p. 206) appears to agree with this. The results communicated in the previous sections also afford strong confirmation. For, if the second cell elongates for any considerable part of the time during which it occupies the position of a second cell, there will be in any random sample of the material a number of second cells which have not reached their full length. This would mean that, on the average, the second cells would be smaller than those further from the apex, and the disparity would be increased if the cells in question continued to lengthen after they had come to be the third, fourth, &c. cells. But this is not so. The average length of all

the second cells measured during the second year was 7·0 units (19·6 μ). The average number of cells in 50 units was 7·3, giving an average length of 6·9 units (19·3 μ). As this includes the apical cell, which is as a general rule rather longer than the second cell, the average length of the cells below the second one is certainly not greater than that of the second cell itself. It may therefore be concluded that the second cell does not lengthen to any measurable extent after it is cut off from the apical cell.

It seems, then, that the normal frequency curve is characteristic of the mature cells. For a complete interpretation of the graphs relating to the apical cell in terms of growth the data are insufficient. Measurement of more cells and at more frequent intervals would seem to be necessary, and it is hoped to undertake this in the near future. Nor has it so far been possible to establish by direct measurement the fact that growth in length is confined to the apical cell, owing to the difficulty of keeping material healthy during a prolonged period of indoor observation.

Some tentative suggestions may be made. A curve such as that for January 16th suggests the presence of a large group of small, probably recently divided, apical cells. That for September 21st shows a similar group, combined with a group of larger cells. Such a curve would result if a large number of apical cells had divided at approximately the same time, and had then grown to a mean length of 8 units before dividing to form a group of new apical cells with a mean length of 5 units. It also suggests that the growth of the apical cell is intermittent, so that two or more groups of apical cells may be in different stages of growth. If this be the true interpretation, then measurement of the apical cells at shorter intervals than a fortnight should afford data demonstrating the passage through the curve of the various groups of cells; it would then be possible to determine the rate of growth.

Some relations between the forms of the curves and the meteorological conditions can be seen. Thus, the smooth curve for May 8th corresponds with a dry period. Similar curves occurred on March 20th, after an exceptionally dry fortnight, and February 13th, after five days, with a total rainfall of 0·1 mm. It is also worthy of mention that a curve similar to that for September 21st was obtained on July 7th, and that both dates followed exceptionally wet periods. But a closer study of the curves reveals many unexplained peculiarities.

(7) *Width of Apical Cell.* The distributions of the widths of apical cells is always approximately symmetrical, and frequently nearly normal. Occasionally the curve tended to be triangular, but there is never any irregularity, and therefore the mean may be taken as a significant measure. The means were calculated from 200 measurements. As the greatest standard error is 0·05, a variation in the mean of 0·1 and more is significant.

The calculated means are shown in Table V, and the graph of their variation in Fig. 12. The graph shows a remarkable seasonal variation. The cells are narrowest in autumn, show a steady increase until the spring,

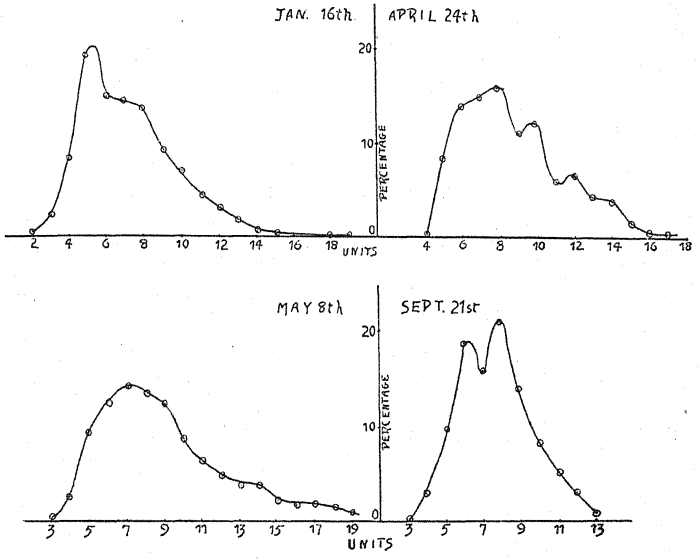


FIG. 11. *Trentepohlia.* Frequency curves for lengths of apical cells.

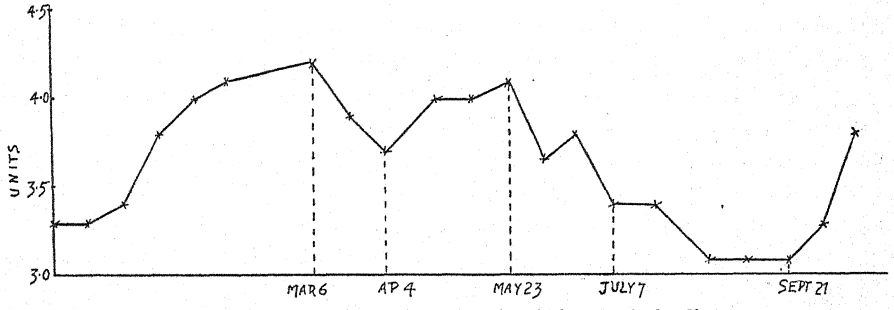


FIG. 12. *Trentepohlia.* Variations in widths of apical cells.

and, after a slight downward fluctuation in April and a second maximum in May, decline fairly steadily again until September. The decline in April does not appear to be correlated with any noticeable weather conditions. Since width of cells is often used in algal diagnosis, this seasonal fluctuation is a feature of some significance.

It is interesting to note that the values for the ratio $\frac{\text{length}}{\text{breadth}}$ of the apical cells are distributed much more regularly than those for the length alone. For example, the material collected on June 9th showed a very

irregular curve for the distribution of lengths. The frequency curve for $\frac{\text{length}}{\text{width}}$ is shown in Fig. 13. If it is continued a little to the left, as shown by the dotted line, it is very nearly normal.

(8) *Mode and Rate of Growth of Cells.* Evidence has already been produced in support of the view that elongation takes place in the apical cell only. Not many apical cells with lengths of less than 4 or 5 units (11·8–14·0 μ) or more than 11 or 12 units were observed. Since the second cells have an average length of 7 units, it appears that, as a general rule, the apical cell grows to a length of about 11 or 12 units (30·8–32·6 μ), and then divides unequally into a second cell of about 7 units (19·6 μ) and a new apical cell of 4–5 units (11·2–14·0 μ). But exceptional cells of lengths as great as 20 units (56 μ) and as short as 2½ units (7·0 μ) have been observed.

Some evidence as to the rate of growth may be obtained by comparing the variation in length of the second cells with the variation in the number of cells in 50 units. Between December 12th and 29th the mean length of the second cell changed from 6·9 to 8·3, or in the ratio of 1 to 1·2. The number of cells in 50 units changed from 7·3 to 6·5, or in the ratio of 1·125 to 1. This indicates that the changed conditions have affected not less than the first four cells, but not as many as six.[1] If growth takes place in the apical cell only, the formation of new cells must have occurred about twice weekly. This period of the year was fairly dry and cold, especially December 29th. In warmer and rainier periods the rate of division appears to be faster. For example, between June 9th and 23rd the mean length of the second cell decreased from 7·0 to 6·3 (19·6–17·6 μ), or in the ratio of 1 to 0·9. The number of cells in 50 units increased in the same period from 7·1 to 8·1, or in the ratio of 0·88 to 1. This seems to indicate that all the cells in the first 50 units are of the new short type. The heavy rains of this fortnight actually commenced on June 10th, so that it appears that as many as seven divisions may have occurred in thirteen days.

On the other hand, attention has already been drawn to the fact that five days of dry weather in the period immediately preceding October 8th, 1927, had been sufficient to produce a long second cell, while cells further from the apex were short. This indicates that only one division had taken place during this period.

This evidence for the rate of growth and its dependence on meteorological conditions is indirect. Direct observation has so far been unsuccessful, no growth having been observed in material kept in a damp chamber.

(9) *Growth of Sporangia.* From November 16th, 1926, onwards the

[1] If the first x cells from the apex are similar to the second cell (length 8·3), and the remaining, $(6·5-x)$ completing the fifty units, are similar to the second cell of a fortnight before (length 6·9), then $8·3\,x+(6·5-x)\,6·9 = 50$, or $x = 3·7$.

size of 400 one-celled branches were measured each fortnight. It was at first difficult to distinguish between cells which were to become sporangia and those which were the beginnings of vegetative branches, but by the

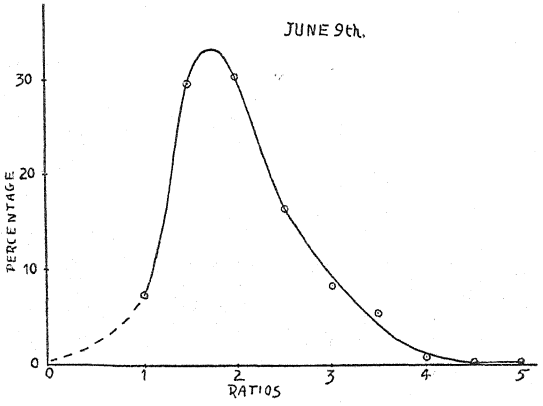


FIG. 13. *Trentepohlia.* Frequency curve for $\frac{\text{length}}{\text{width}}$ of apical cell.

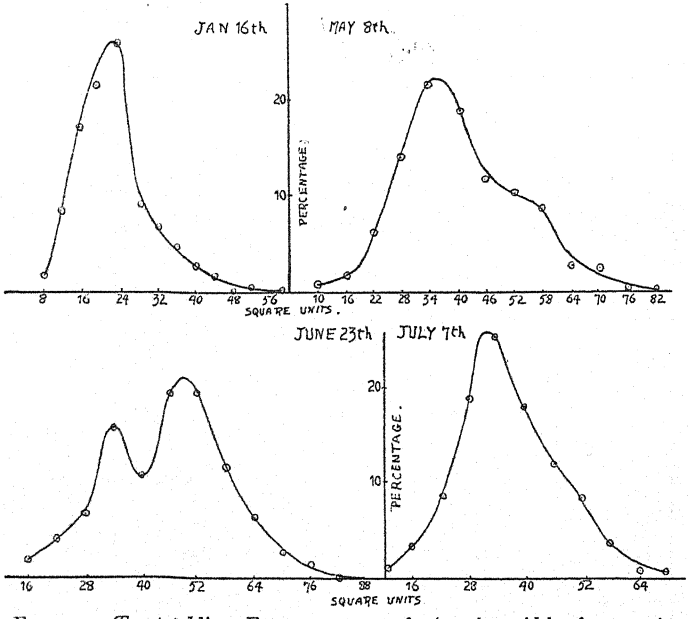


FIG. 14. *Trentepohlia.* Frequency curves for length × width of sporangia.

end of December the sporangia were clearly distinguishable by their rounder form, and from this time onwards these alone were measured. As already stated the development of the sporangia is spread over a long period, during which there is more or less progressive enlargement. As the cells are approximately ellipsoidal the area of their elliptical section afforded the

most convenient measure of size. The product of the greatest and least diameters was taken as giving a value which is proportional to the area (area times a constant ratio). The distribution of the areas about their mean was fairly regular but not symmetrical. Sometimes a second peak appeared, suggesting a second group of sporangia at a different stage of development, but it was always possible to distinguish the main group. Fig. 14 gives the curves for January 16th, May 8th, June 23rd, and July 7th. These curves are drawn after the measurements have been collected into groups. In measuring a cell with dimensions 8×6 units, errors from $\frac{1}{4}$ to $\frac{1}{2}$ a unit in each dimension are possible. Such errors would result in an error of 4 to 6 in the product. Unless, therefore, measurements differing by less than this are grouped together, a number of products will fall into the wrong group and an artificially irregular curve will result. Until February 13th groups of four were considered sufficient. From March 6th groups of six were used. Thus on this date all the cells given as of area 40 include those ranging from $37\frac{1}{2}$ to $42\frac{1}{2}$, and half of those measured as 37 or 43.

The curves being unsymmetrical the mean is not as significant as the mode, since the variations of the mode indicate the changes in the predominant type of cell. The values of the mean and mode are given in Table VI, and are shown graphically in Fig. 15.

TABLE VI.

The Means and Modes (with their Logarithms) of the Length × Breadth of Sporangia.

Date.	Sporangia.			
	Mean.	Log. Mean.	Mode.	Log. Mode.
Nov. 15, 1926	9·2	0·964	8·4	0·924
„ 27, „	15·8	1·199	14·3	1·155
Dec. 12, „	17·0	1·230	16·0	1·204
„ 29, „	18·8	1·274	19·0	1·279
Jan. 16, 1927	23·1	1·364	22·8	1·358
„ 31, „	25·8	1·412	24·0	1·380
Feb. 13, „	26·5	1·423	25·2	1·401
Mar. 6, „	33·8	1·529	28·8	1·459
„ 20, „	34·0	1·531	30·0	1·477
Apr. 4, „	40·4	1·606	37·0	1·568
„ 24, „	40·4	1·606	36·0	1·556
May 8, „	40·3	1·605	36·5	1·562
„ 23, „	41·1	1·614	44·0	1·643
June 9, „	43·2	1·635	41·2	1·615
„ 23, „	44·9	1·652	49·0	1·690
July 7, „	36·4	1·561	33·0	1·519

Growth of the sporangia has been found to take place logarithmically. The logarithms of the successive modes and means have been plotted in Fig. 15, B. The points corresponding to the means are scattered irregularly, but those corresponding to the modes are, with the exception of the first three, grouped along a straight line. The first three are of doubtful signifi-

cance, since they were measured at a time when the differentiation between sporangia and true branch cells was not clear. When distinction became possible, the sporangia were much rounder and larger than the other cells.

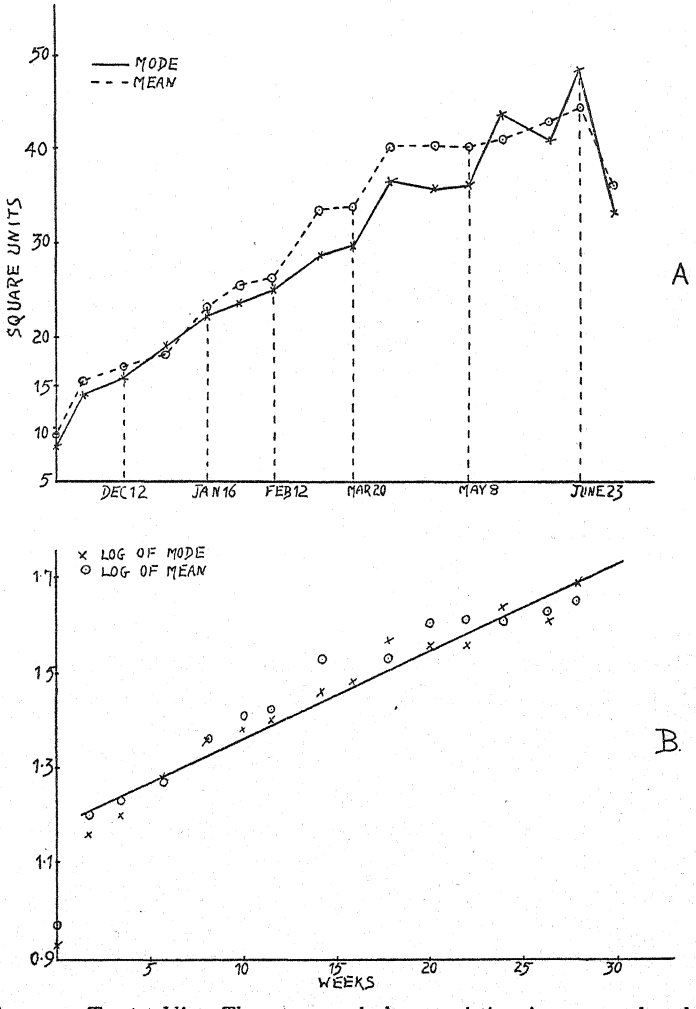


FIG. 15. *Trentepohlia.* The upper graph shows variations in means and modes of areas of growing sporangia; the lower graph shows the logarithms of the same.

Therefore it is probable that the first three samples taken had too low an average area, and this is what the graph suggests. The equation of the line has been found. If A is the area of the sporangia in square units and t the time in weeks after November 15th, 1926, the equation of growth is

$$\log_{10} A = 1{\cdot}2 + 0{\cdot}015t$$
$$= \log_{10} 15{\cdot}85 + 0{\cdot}015t$$
$$\text{i.e.} \quad A = 15{\cdot}85 \times 10^{0{\cdot}015t}$$

This gives the general tendency of the growth. The departures from the straight line in Fig. 15, B, may represent irregularities due to external conditions. April 4th shows growth considerably in advance of expectation, and this follows a very wet period; while the retarded growth of May 8th is after a dry period. As soon as the rain comes after this, the growth is very fast, exceeding the normal. After unusually fast growth there is usually a period of slow growth, as in the periods from April 4th to May 8th and from May 23rd to June 9th.

The graph for June 23 (Fig. 14) may be due to some of the larger sporangia having dehisced. There is still a predominance of large cells, but not enough to obscure the presence of a smaller group of less-developed cells which were not evident in the earlier curves. By July 7th only the small cells remain, as nearly all the large sporangia have emptied. On this date there is a smooth distribution of approximately normal type, except for a slight irregularity on the right (Fig. 14), which is probably due to a few large ripened sporangia which had not emptied. It is clear that the distribution of June 23rd is the sum of two distributions, each of approximately normal type and affording evidence of the development of two groups of sporangia which ripen and dehisce successively.

(10) *Branching.* Every fortnight the number and size of the branches occurring on the first ten cells of 200 filaments were estimated. Some connexion between meteorological conditions and increase or decrease of branches was expected, but it was found that the results were only definite as regards one-celled and two-celled branches. Many of the former represent sporangia, and the apical cells of two-celled branches are also often sporangia. The number of these branches decreased considerably at the end of July. On May 23rd the number of one-celled branches on the first ten cells of 200 filaments was 330, on July 26th, 140. On May 23rd the number of two-celled branches was 83, on July 26th, 36. But as soon as the sporangia had set free their zoospores, new branches were rapidly formed, many of which were potential sporangia for 1928. On September 5th the number of one-celled branches was 297, of two-celled, 71.

Summary.

1. The moisture relations of *Trentepohlia aurea, Mart.* have been studied by direct observation and experiment and by statistical analysis of measurements of the cells.

2. The alga is able to resist drought for long periods, and survived in a desiccator for six months. The dry material can reabsorb water rapidly. Comparison of the rates of drying and reabsorption with those of non-living material suggest that the water used by the alga is retained by the walls and between the threads.

3. High salt concentrations are necessary to effect plasmolysis of the cells. Desiccation increases the resistance to plasmolysis. The cells are less readily plasmolysed in spring and summer than in autumn and winter.

4. The following measurements were made on samples of the alga collected each fortnight:

(*a*) Lengths of 400 apical and 400 second cells.
(*b*) Widths of 200 apical cells.
(*c*) The lengths and breadths of 400 sporangia.
(*d*) The number of cells in a definite length (140 μ) measured from the tip of the apical cells of 200 filaments.

5. Growth appears to be confined to the apical cell.

6. The sizes of cells which have ceased to grow vary about their mean according to a normal law, but the distribution of the lengths of growing cells is irregular.

7. The width of the apical cell is greatest in spring and least in autumn, independently of meteorological conditions.

8. Shorter cells are formed in damp and warm weather, when growth is more rapid.

9. The growth of the sporangia is logarithmic, with variations corresponding to moisture conditions.

I am indebted to Professor F. E. Fritsch for the suggestions from which both parts of this paper were evolved and for frequent advice. I have also to thank Mr. R. C. J. Howland for instruction in statistical methods and for assistance in interpreting the results.

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NOTE.

SECRETORY CELLS IN A FOSSIL LEAF.—In the course of an investigation of Eocene leaf-cuticles (carried out with the aid of a grant from the Royal Society), it was found that the lauraceous leaf, *Litsea bournensis*, almost invariably has numerous dark spherical bodies apparently attached to the cuticle (see Fig. 1); they are especially abundant where it has been folded, and are also found floating in the

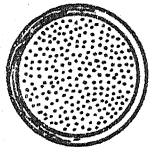


FIG. 1.



FIG. 2.



FIG. 3.

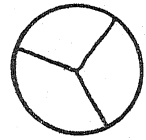


FIG. 4.



FIG. 5.

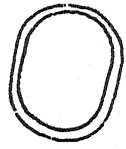


FIG. 6.



FIG. 7.

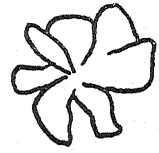


FIG. 8.

FIGS. 1–8 illustrating the secretory cells in a fossil leaf.

liquid used for clearing the leaf. Their appearance at first suggests an epiphytic fungus or alga, and moreover they frequently show internal segmentation into two, three, or four portions resembling spores (see Figs. 2–5). Their true nature, however, was revealed by the careful microchemical tests made by Mr. L. A. Boodle to whom I had submitted them, and to whom I owe grateful thanks. The spherical bodies remain unaffected on treatment with the strongest acid or with Eau de Javelle, but they take up stain readily. They are insoluble in alcohol, but on treatment with chloroform become transparent, and then show a cuticularized wall from which the contents dissolve out (see Fig. 6).

The apparent segmentation of the contents is due to their hard brittle nature, and can be brought about by pressure on the coverslip. By reflected light they appear brownish yellow with a dark periphery. Solereder points out that in the mesophyll of various Lauraceae, secretory elements occur in the form either of oil cells or of mucilage cells, or both. Bokorny (Flora, lxv, pp. 339–50, 1882) has described these secretory cells in detail. In *Litsea*, the cells containing resin, wax, or oil, vary in diameter from 0·2 to 0·6 mm., and are spherical or ellipsoidal with large cavities and narrow walls, whereas the mucilage cells have a different shape and an extremely narrow lumen.

The bodies present in the fossil were compared with the oil cells of the recent *Litsea stocksii* and others, and found to agree with them very closely both in form and in their reaction to microchemical tests. The contents have become hard and brittle on fossilization (see Figs. 7, 8), but otherwise seem to be unaltered.

Their original position is in the mesophyll from which they are set free on maceration, tending then to adhere to the cuticle by capillarity.

Similar bodies have been found in the fossil leaf *Neolitsea gardneri*, also from the Bournemouth Eocene.

The occurrence of these resin cells in Eocene leaves seems worthy of record, and a warning as to the possibility of confusion with some epiphytic thallophyte may be useful to other workers.

H. BANDULSKA.

Studies on the Transport of Nitrogenous Substances in the Cotton Plant.

I. Preliminary Observations on the Downward Transport of Nitrogen in the Stem.[1]

BY

E. J. MASKELL

AND

T. G. MASON.

With eight Figures in the Text.

THE current view appears to be that, though the bulk of the nitrates absorbed by the roots reaches the leaves and is there synthesized to higher forms of nitrogen, yet other parts of the plant may also receive nitrogen in the form of nitrates, and may synthesize amino-acids and proteins directly. Thus, according to Czapek (5), the predominating importance of foliage leaves in the building up of protein in green plants can scarcely be considered a matter of doubt to-day, though it seems equally certain that other organs possess the capacity of sharing in a definite degree in the formation of protein. In the foliage leaves the most favourable conditions for protein synthesis occur, inasmuch as there we find the most abundant access of nitrogen compounds through the transpiration current, as well as the most abundant supply of sugar.[2]

This view, that the bulk of the mineral nitrogen absorbed by the plant is elaborated in the leaf, assumes that there is a movement of nitrates into the leaf from the *wood*, and, it would seem, a corresponding export of organic nitrogen, presumably via the phloem. It will be evident that this ingress and egress of nitrogen are interdependent, for an export of organic nitrogen from the leaf must involve a corresponding import of inorganic nitrogen from the soil. Now the only claim the leaf would appear to have on such a supply of nitrates rests on its great transpiration pull. If nitrates

[1] Paper No. 3, from the Physiology Department of the Cotton Research Station, Trinidad.

[2] In the cotton plant, however, the concentration of sugar in the leaf lamina was found to be less than in any other part of the plant examined, except the xylem.

[Annals of Botany, Vol. XLIII. No. CLXX. April 1929.]

were to travel from the roots in the sieve-tubes by a process analogous to diffusion, then the leaf would have no greater pull on the supply of nitrates absorbed from the soil than any other organ, unless of course the nitrate gradient into the leaf was steeper than that into other organs. As this is improbable, for there seems to be a consensus of opinion (1, 6, 9, 13) that little or no nitrates occur, for instance, in fruits and seeds, it ought to follow that the nitrates imported by the leaf enter via the wood and not via the bark.

Curtis (4) has recently impugned the hypothesis that inorganic nitrogen moves with the transpiration current in the wood. In doing so, it seems to us, he also casts doubt on the view that the leaf is the main factory of protein synthesis and that there is an export of organic nitrogen from it. If it were demonstrated that there is normally an export of organic nitrogen from the leaf, the view that the nitrates travel in the xylem would, we think, be greatly strengthened.

Chibnall (3) has established a strong probability that there are diurnal variations in the nitrogen content of the leaf, the total nitrogen increasing by day and diminishing by night. He has legitimately argued that this indicates an export of nitrogen from the leaf during the night. His conclusion that there is a loss of nitrogen from the leaf by night depends, however, very largely on whether he is justified in expressing his results on the fresh weight basis. As some doubt may exist as to the validity of the assumptions on which this method of expressing the results depends (cf. 8), a withdrawal of nitrogen from the leaf is still perhaps in doubt.

Curtis (4) has endeavoured to settle directly the question of the path taken by nitrates by means of ringing experiments. He has found that removal of a ring of bark 'distinctly hinders the movement of nitrogen and of ash constituents into the leaves above the ring'. As he himself recognized, if nutrients are carried in the transpiration stream, a check in transpiration from the leaves above the ring would reduce the movement of solutes into them. As his analyses were not made for a number of weeks after the operation of ringing, the occurrence of morbid changes in the wood leading to a reduction in the transpiration current is rendered probable. In fact he states 'I have obtained considerable evidence showing that transpiration from the leaves of the ringed stems is distinctly less than that from normal leaves'. 'In order to eliminate this factor of transpiration', he carried out an experiment involving the use of ringed trees only. Instead of comparing the nitrogen content of a ringed twig with that of a normal twig, he compared the nitrogen content of a defoliated region of stem having a ring at its base with the nitrogen content of the corresponding region of stem having a ring at its apex. He found that 'the rings at the bases of the defoliated parts distinctly reduced both their total nitrogen content as well as that per gram of dry weight'. Curtis considered

that the results indicate 'that the low nitrogen content above a ring is not due to a lessened transpiration'. As he states, however, 'the data cannot be considered as conclusively proving that nitrogen[1] or other nutrients move upward primarily through the phloem and not through the xylem'.

Curtis's data, as he himself recognized, might be explained on the assumption that nitrates are combined with carbohydrates in the lower part of the plant, possibly in the roots (10, 12), and move upwards as organic compounds in the phloem. A possibility not referred to is that elaborated nitrogen may be synthesized in both roots and leaves, and may be exported from both these regions. It is conceivable that the bulk of the nitrates absorbed during the day may reach the leaves with the transpiration current, and that the nitrates absorbed by night, while the rate of transpiration is low, may remain in the roots and be there elaborated into amino-acids, &c.

The work reported in the present paper was done on the dried material obtained in the course of our studies on the transport of carbohydrates (7, 8). It was considered that an examination of the variations in total nitrogen content of the tissues would pave the way for a more detailed study of the problem in which the individual water-soluble fractions could be analysed separately and their actual concentrations determined.

Methods.

As an account of the methods employed in collecting and handling the material has been already given (7), we need only outline the more important facts here. Vegetative branches were removed about a month after planting, so that the scaffolding consisted only of the main axis and its fruiting-branches. With the exception of the second series of diurnal observations, the plants were without bolls as the flower-buds were cut off before the time of flowering. The salicylic-thiosulphate method as modified by Ranker (11) was used for the determination of total nitrogen.

Expression of Results.

The observations recorded are in all cases the means of two or three samples. The statistical treatment is essentially the same as that employed in studying the transport of carbohydrates. Standard deviations due to sampling are shown graphically on the right of the figures.

The results are expressed both on the fresh weight basis and on the basis of residual dry weight (dry weight less total carbohydrates). The former method is that generally employed and, as has been shown by Chibnall (3), is certainly more accurate than the dry weight basis when

[1] From the context it is clear that 'nitrogen or other nutrients' refers to inorganic compounds absorbed by the roots from the soil.

diurnal changes in the normal plant are under investigation. Its use depends on the belief that variations in the water content of a leaf or other mature organ are normally relatively small, and are approximately balanced by concomitant variations in carbohydrates. The method assumes that the fresh weight of an organ will be approximately constant in time. It is clear, however, that the method cannot be applied to cases in which plants are differentially treated in any way which might affect their moisture relations. The objection to the dry weight basis is that the dry weight of an organ, and in particular of a leaf, is by no means constant in time, but varies considerably owing to the accumulation of carbohydrates by day and their disappearance by night. The greater part of the variation in dry weight of a mature organ such as a leaf over a short period of time is undoubtedly due to variation in carbohydrates (sugars and reserve carbohydrates[1]) and the remaining fraction of the dry weight—residual dry weight—is relatively constant. The residual dry weight has accordingly been suggested as a basis for the calculation of results (7). The two methods have been discussed in greater detail in the previous paper. It was shown in that paper that, whereas the net variation in time of the fresh weight was considerably smaller than the variation of the dry weight, the residual dry weight showed a smaller variation still. In general, therefore, the residual dry weight basis seems preferable and, in the case of ringing experiments, it appears to be the only basis that can legitimately be used.[2]

1. *Diurnal Variations in Total Nitrogen of Leaf and Bark.*

(a) *Diurnal Series 1.*

Procedure. In the first series of observations, collections of leaves, bark, and wood were made at intervals of two hours. The first collection was made at 5.30 p.m. on the 31st of August, and the last at 3.30 p.m. on the following day. Each collection consisted of two samples, and each sample was taken from ten plants. The leaf samples consisted of thirty mature leaves, three from the top of the main axis of each plant; the larger veins were removed and rejected. Bark and wood were taken from about six inches of the stem below the first fruiting-branch.

Results. The results for leaf and bark, expressed as the weight of total nitrogen per 100 grm. residual dry weight, are shown graphically in

[1] In our experiments sugars are estimated by analysis of the expressed sap and the reserve carbohydrates by acid hydrolysis of the dried material.

[2] The above discussion refers, of course, only to those cases in which it is necessary or desirable to reduce the data obtained for each sample to some standard basis. The ideal method is to use samples of such a size that the weights per sample shall be sufficient in themselves. Samples of the required size are, however, rarely practicable when changes over a short time interval are under investigation.

the lower part of Fig. 1. The results are also expressed per 100 grm. fresh weight, and will be seen in the upper part of the figure. In order to exhibit

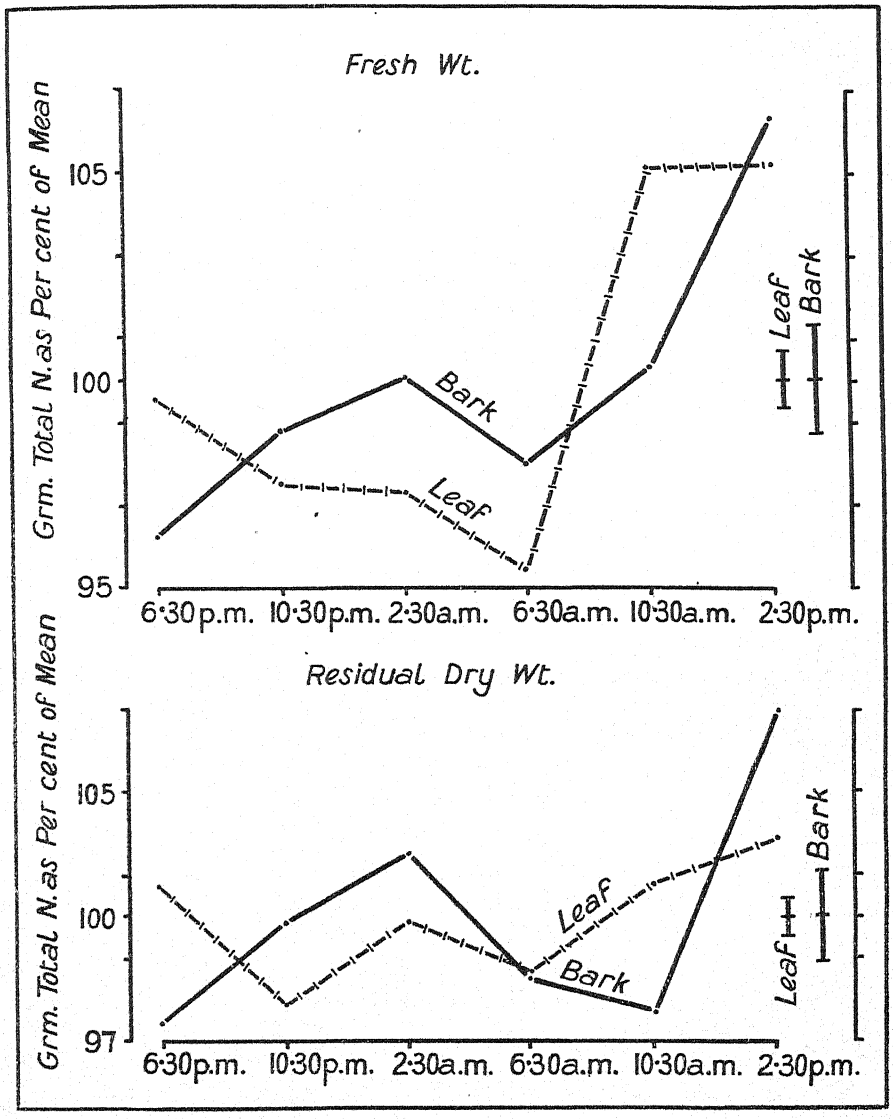


FIG. 1. Diurnal variations in the total nitrogen content of leaves and bark (Series 1). Results expressed per 100 grm. fresh weight (upper) and per 100 grm. residual dry weight (lower). Plotted as a percentage of the mean value for each tissue. Standard deviations due to sampling shown by the vertical lines on the right.

the general drift in time, the actual observations have been smoothed by taking the means of successive pairs of observations, and in order to show

the relative variations in the two tissues, the smoothed values are expressed as a percentage of the mean value for each tissue.

It will be noticed that, when expressed on the fresh weight basis, the diurnal variation in nitrogen content of the leaf and bark follows a rather definite pattern. The nitrogen of the leaf falls throughout the night but rises rapidly in the morning. In the bark there is a gradual accumulation of nitrogen during the night and the early morning; a drop then occurs which is followed by a very marked increase in nitrogen content. This increase takes place a little later than the rise in the leaf. Taking the paired observations as plotted, we find that in the leaf the standard deviation due to sampling (1·43 per cent.) is markedly less than the observed standard deviation in time (4·17 per cent.), so that there is definite evidence of variation in time. In the bark, however, the observed standard deviation in time (3·56 per cent.) is not much greater than that due to sampling (2·65 per cent.). Statistically, therefore, there is no real evidence of variation in time in the nitrogen content of the bark tissues. The correlation coefficient, however, between the nitrogen content of the leaf and bark amounts to +0·603, and is moreover statistically significant (P = 0·04). As the leaf variation in time is in itself statistically significant and is significantly correlated with the variation in the bark, it might be inferred that there is real variation in time in the nitrogen content of the bark, but the very small excess of the observed variation in the bark over that which might have been due to sampling renders the inference doubtful.

Inspection of the curves suggests not only a direct correlation between leaf and bark, but also that changes in the bark tend to lag behind those in the leaf. Advancing the leaf values a single period might therefore be expected to augment the value of the correlation coefficient. When this is done the correlation coefficient is increased to +0·766. This is what occurred in our study of the diurnal variations of the sugar concentrations of leaf and bark. It suggests that possibly *concentration* changes in the leaf lead to similar changes in the *concentration* of diffusible nitrogenous substances in the bark sap, and that consequently physical diffusion may be a factor in the transport of organic nitrogen from the leaf. In view, however, of the fact that these results are obtained on a fresh weight basis, and that the evidence for real variation in time of nitrogen content in the bark is doubtful, the suggestion must be a very tentative one.

Turning now to the results expressed per 100 grm. residual dry weight, it will be observed that though the night values for the leaf are on the average less than those for the day, the difference between the day and the night values is less marked than when the results are expressed on the fresh weight basis. In the case of the bark, the accumulation of nitrogen by night is more marked than it is on the fresh weight basis. Moreover, the differences between the day and night values are smaller. In the case

of the leaf, the standard deviation due to sampling (0·92 per cent.) is exceeded by that observed in time (1·46 per cent.), but the difference is not quite significant. In the case of the bark, the two standard deviations are still closer to one another, 2·29 per cent. for sampling and 2·86 per cent. for the variation in time. There is thus some evidence of variation in time in the nitrogen content of the leaf, whether the results are expressed on the residual dry weight or fresh weight basis. In the case of the bark this is not the case.

To sum up, the night values for the total nitrogen of the leaf are less than the day values by both methods. Our results therefore confirm Chibnall's findings and indicate that there is a withdrawal of nitrogen from the leaf during the night. The evidence for diurnal variation in the bark is less satisfactory. On the fresh weight basis it would appear that there may be diurnal variations, and that changes in the nitrogen content of the leaf are followed by similar changes in the bark. On the residual dry weight basis the correlation (+0·234) between leaf and bark is not significant and is not augmented by advancing the leaf values.

(b) *Diurnal Series 2.*

Procedure. In the second series of observations, collections were made at intervals of three hours. The first collection was made at 6 a.m. on the 16th of March, and the last at 6 p.m. on the 17th of March. Each collection consisted of two samples, and each sample was taken from twenty plants. As before, three mature leaves from near the top of the main axis were taken from each plant. The bark was taken from six and a half inches of stem below the first fruiting-branch. The plants were bolling freely at the period the observations were made.

Results. The results, smoothed as in the first series, are shown graphically in Fig. 2. It will be seen that the diurnal variation in the nitrogen content of the leaf follows a similar course by whatever method the results are expressed. On the fresh weight basis the curve is of a much more regular nature. The total nitrogen content of the leaf again increases during the day and diminishes during the night. There is no indication, even on the fresh weight basis, of diurnal variation in the nitrogen content of the bark. The cause of this is possibly to be found in the fact that the plants in this case were bolling freely. Murneek (9) has shown that in the tomato the fruits monopolize the nitrogen of the plant. If a similar state of affairs prevails in cotton, as seems probable, the bulk of the elaborated nitrogen might travel from the leaf to the boll and little to the roots. The bark examined was taken from the main axis below the fruiting-branches, and would not necessarily, therefore, exhibit appreciable diurnal variations in nitrogen content.[1]

[1] It is interesting to note that the amount of nitrogen associated with 100 grm. residual dry

The statistical data are shown in Table I.

TABLE I.

Standard Deviation due to—	Leaf. Fresh Weight.	Leaf. Resid. Dry Weight.	Bark. Fresh Weight.	Bark. Resid. Dry Weight.
Sampling	1·35	1·31	2·23	2·19
Time + Sampling	5·41	5·23	1·88	1·37

It will be observed (Table I) that there is again evidence of variation in time in the case of the leaf. It may therefore be concluded that there is a withdrawal of organic nitrogen during the night from it. In the case of the bark, however, the observed variation in time is less than would be expected from the variation due to sampling, and there is no evidence therefore of any real change in nitrogen content of the bark during the experiment.

2. *The Effect of Ringing the Stem* (Experiment 1 (7))[1]

Procedure. Three days before the commencement of the experiment a region of stem, two feet long, at the base of the plant was cleared of all leaves and branches. A piece of tape was then tied round the stem at a distance of one foot from the soil. At dawn on the day of the experiment half the plants were ringed at the level marked by the ring of tape, the actual operation being completed in less than one hour. The width of the ring of bark removed was half an inch. The exposed wood was water-proofed with vaseline.

The material for analysis was taken at intervals of approximately six hours, commencing with a collection of Normal (unringed) plants at 6.45 a.m. on the 28th of September. The stem samples consisted of the seven inches of stem above and below the ring (Ringed plants) or tape (Normal plants). It should be noted that the portion handled lay on the track from leaves to root. In the laboratory the pieces of stem were trimmed to a length of six and a half inches from the ring or tape as the case might be. The bark and wood from each sample were separated and weighed. Three mature leaves were taken from near the top of the main axis of each plant. There were two samples per collection, each of ten plants.

Results. The results of the analyses of the leaves of the two groups are expressed per 100 grm. residual dry weight, and per 100 grm. fresh

weight in the bark of Diurnal Series 2 is 12·65 per cent. less than that in the bark of Diurnal Series 1, whereas the amount of total carbohydrates is 26·8 per cent. greater.

[1] The numbers of the experiments are those of the two papers on Transport of Carbohydrates (7, 8).

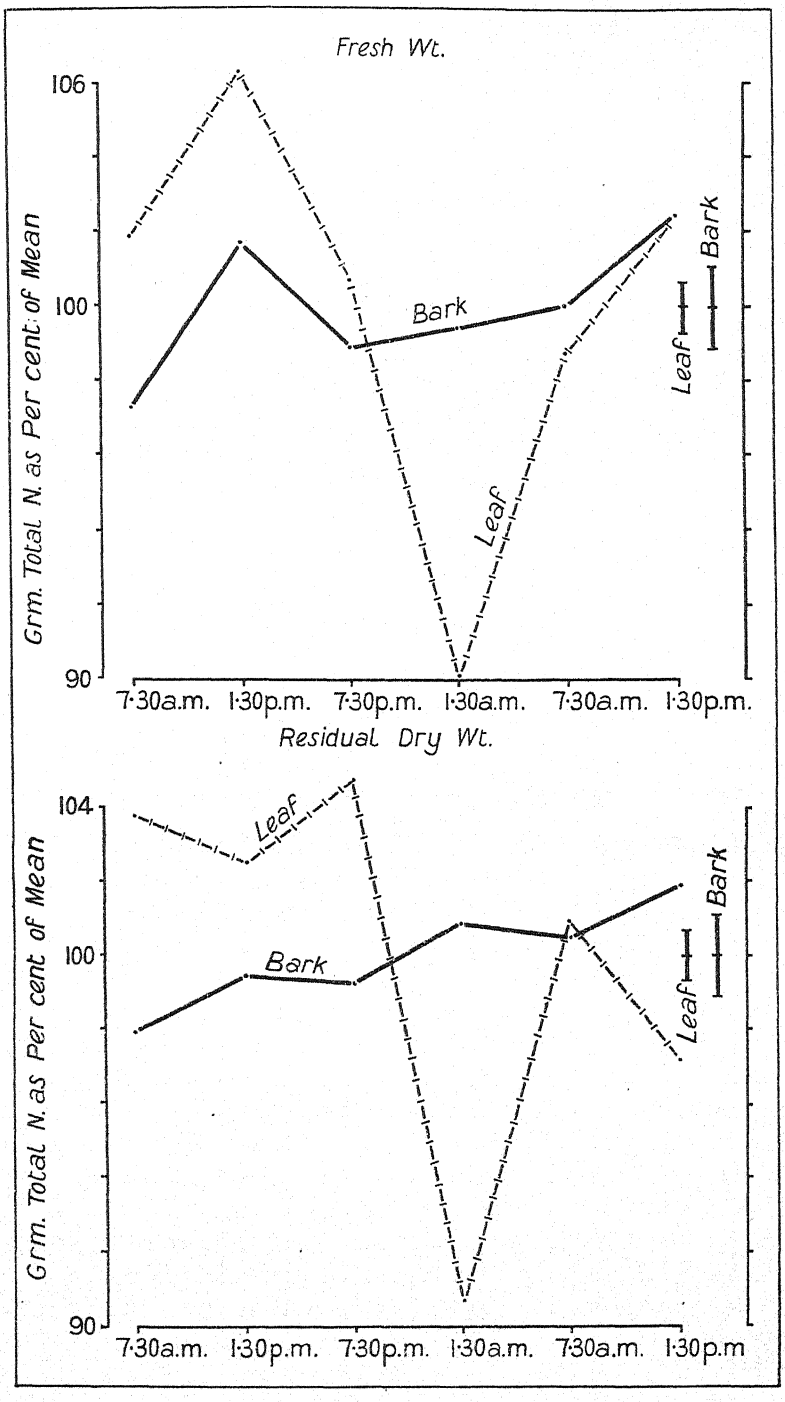


FIG. 2. Diurnal variations in the total nitrogen content of leaves and bark (Series 2). Results expressed per 100 grm. fresh weight (upper) and per 100 grm. residual dry weight (lower). Plotted as a percentage of the mean value for each tissue. Standard deviations due to sampling shown by the vertical lines on the right.

weight. Inspection of the curves (Fig. 3) will make it clear that there was but little difference in nitrogen content between the two groups of plants. On the fresh weight basis, the leaves of the Ringed plants do, however, exhibit a higher nitrogen content during the night. This is due to the fact that the leaves of the Ringed plants failed to recover their normal moisture

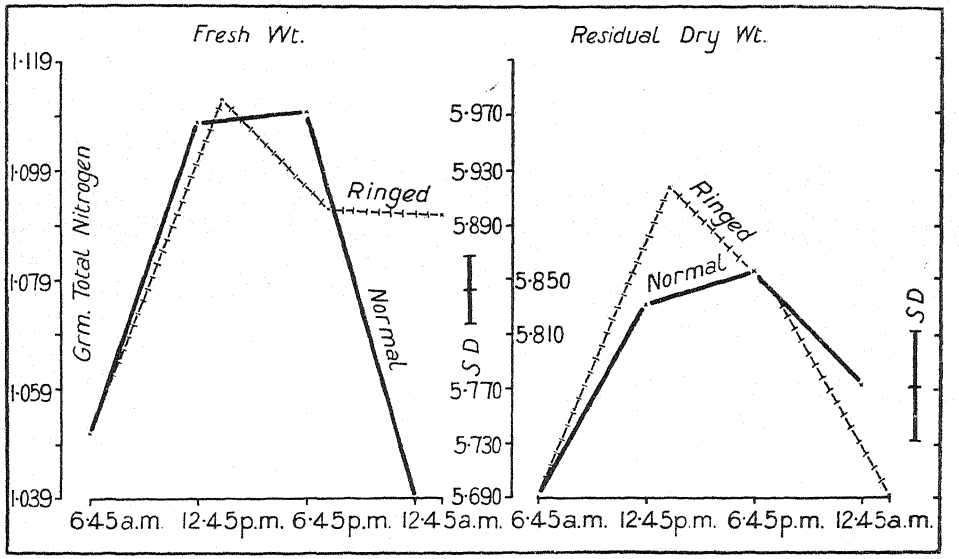


FIG. 3. Weight of nitrogen per 100 grm. fresh weight (left) and residual dry weight (right) in leaves of Ringed and Normal plants. S.D. = Standard deviation due to sampling.

content at night (cf. 7, p. 35). The results obtained on the fresh weight basis are, therefore, liable to be distorted, and greater weight must be given to the results obtained on the basis of residual dry weight. On this basis the leaves of the Ringed plants show a higher nitrogen content about mid-day, but the excess over the leaves of the Normal plants is too small to be regarded as significant. The observed differences between the Normal and the Ringed plants fall well within the limits of sampling variation.

The sequence of change in both groups of plants has evidently followed the normal course of events, the nitrogen content rising by day and diminishing during the night. The rise by day in the Ringed plants indicates that nitrates traversed the stem in the wood. The actual increase in nitrogen in the Ringed plants per 100 grm. fresh weight between 6.45 a.m. and 1 p.m. was 0·061 grm. As the standard deviation due to sampling was only 0·0125 grm., a rise of 0·061 grm. is easily significant ($P = 0{\cdot}015$). When the results are expressed per 100 grm. residual dry weight, the increase amounts to 0·226 grm., while the standard deviation is 0·0831. As the actual rise is nearly three times the standard deviation ($P < 0{\cdot}1$), it seems probable that the leaf continued to gain nitrogen after the removal of the

bark. The point to stress is that, whether the results are expressed on the fresh weight or on the residual dry weight basis, the increase in nitrogen content of the leaf appears to be significant. It may therefore be concluded that removal of the bark tissues has not interrupted the movement of nitrates through the stem, and consequently that the channel of nitrate transport is the wood.[1]

Next we consider the nitrogen content of the bark tissues *above* the ring (Ringed plants) or tape (Normal plants). The results, which are shown in Fig. 4, are expressed as the weight of nitrogen in the bark from six and a half inches of stem. They are calculated both on the mean residual dry weight and on the mean fresh weight basis. It will be seen that in both groups and on both bases of expression the nitrogen content increased during the day and diminished by night.

The significance of this apparent diurnal change may be tested by comparing the mean of the two collections at midday and at evening, with the mean of the two collections at dawn and at about midnight. On the residual dry weight basis the excess shown by the midday and evening collections is 0·0088 grm. for the Ringed plants, and 0·0041 grm. for the Normal plants, while the standard deviation is 0·00262 grm. In the case of the Ringed plants at least, therefore, the diurnal change is fully established ($P = 0{\cdot}015$).

It will be noticed also that the Ringed plants show an accumulation of nitrogen in excess of the Normal plants. Calculating the results for the mid-points in time between the Ringed and the Normal collections, we find an average excess in the Ringed plants of 0·00454 grm. The standard deviation is 0·002137, so that the excess is probably significant (P lies between 0·05 and 0·10). The removal of the ring of bark, while apparently preventing the downward migration of nitrogen past the ring, has not prevented transport from the leaf into the bark above the ring.

In the bark *below* the ring (lower part of Fig. 4) there appears in the Normal plant a diurnal change similar to that in the bark above. Also the Ringed plants have on the average a smaller nitrogen content than the Normal plants. (The mean difference, calculated for mid-points, is 0·00307 grm.) The sampling variation is, however, greater than in the bark above the ring, and neither the apparent diurnal change in the Normal plant nor the mean difference between Ringed and Normal is statistically significant.

The results for the wood are shown in Fig. 5. It will be noticed that the wood of the Normal plant does not show the increase by day and decrease by night shewn by the bark. Above the ring, the Ringed plants contained more nitrogen than the Normal plants did above the tape. This

[1] The increase in nitrogen content of the leaves is not due to a depletion by the leaves of the stem tissues above the ring, for these tissues, as will be shown, also increase in nitrogen content.

is indicated by both methods of expressing the results. From this it would appear that there is not only a downward movement of nitrogen through

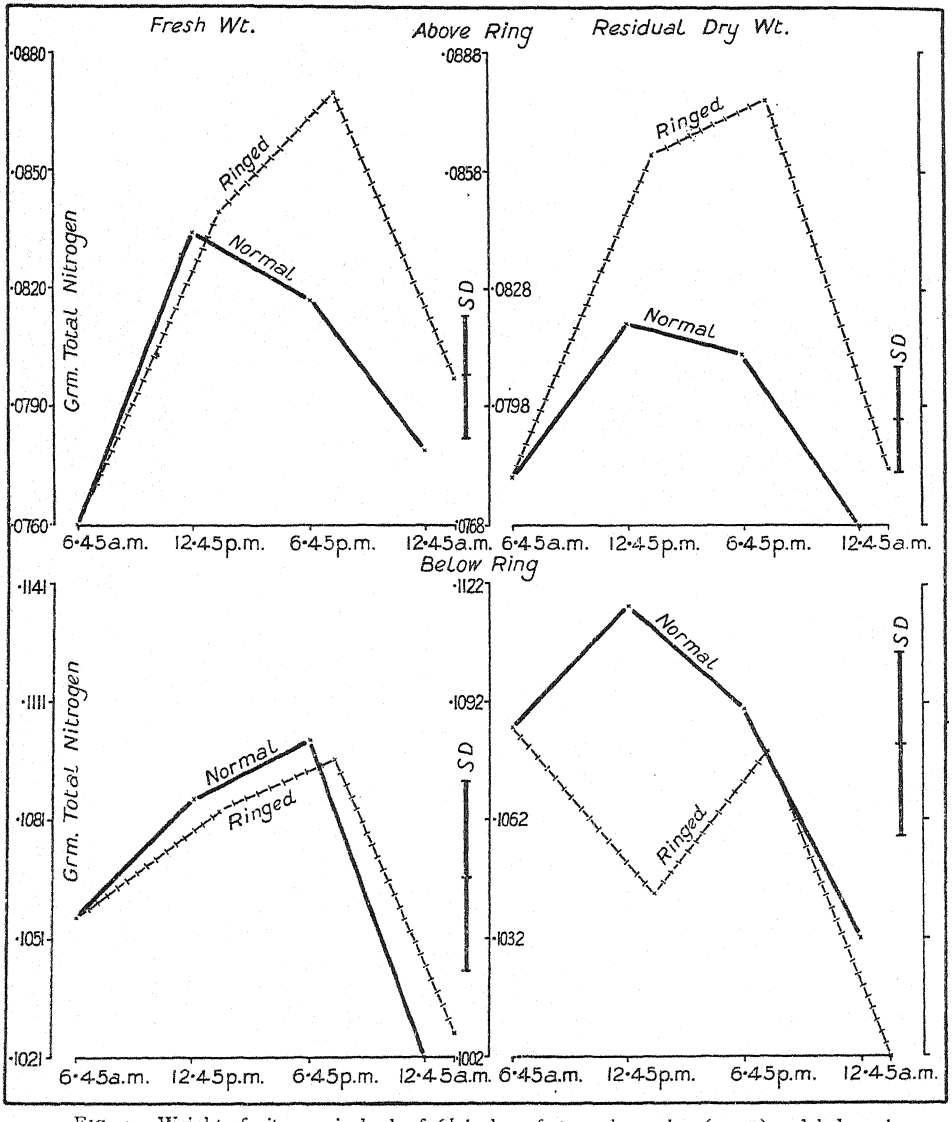


FIG. 4. Weight of nitrogen in bark of 6½ inches of stem above ring (upper) and below ring (lower). Results expressed on mean fresh weight (left) and mean residual dry weight (right). S.D. = Standard deviation due to sampling.

the bark,[1] but also a horizontal movement from bark to wood. It will be noticed that the sampling variation is relatively small, and that the excess

[1] It is shown subsequently (p. 220) that appreciable quantities of organic nitrogen are not transported downwards in the wood.

of the Ringed over the Normal plants is generally many times the standard deviation. *Below* the ring the Ringed plants contain less nitrogen than the

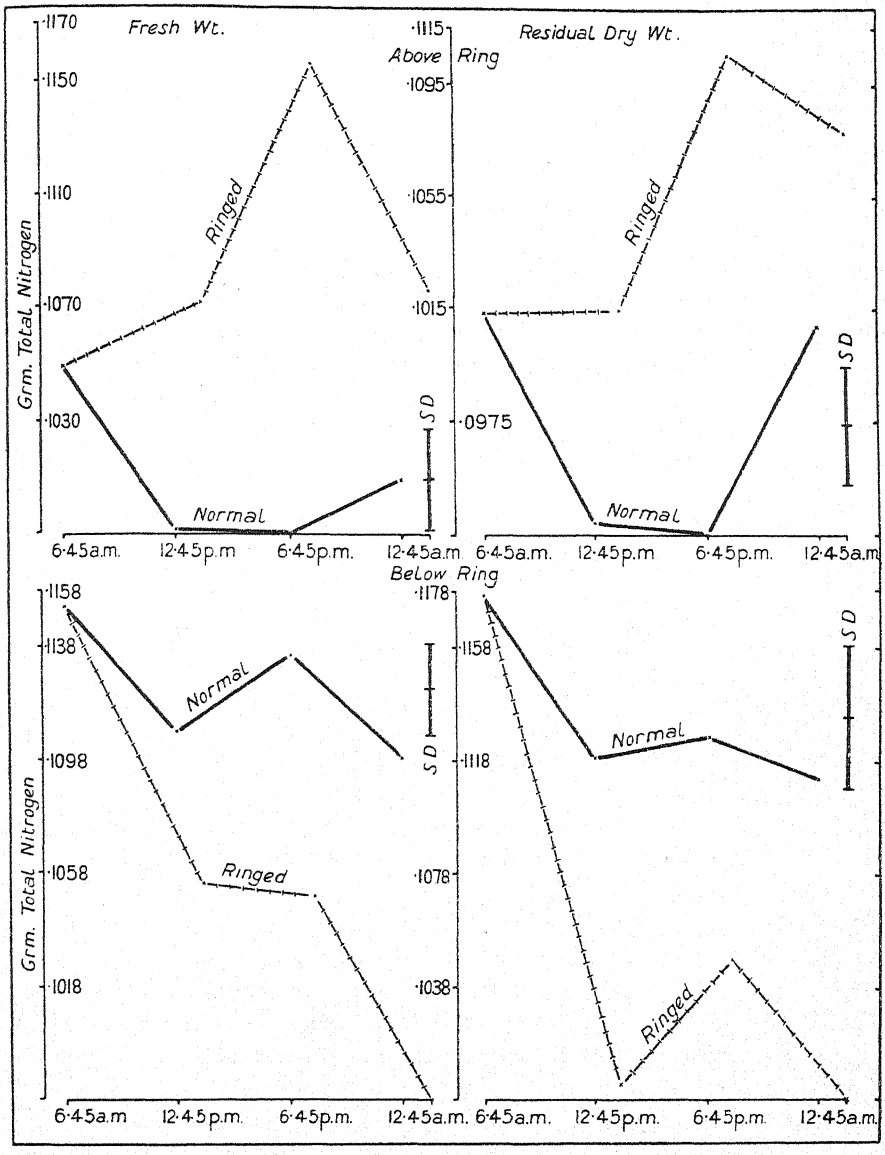


FIG. 5. Weight of nitrogen in wood of 6½ inches of stem above ring (upper) and below ring (lower). Results expressed on mean fresh weight (left) and mean residual dry weight (right). S.D. = Standard deviation due to sampling.

Normal plants, indicating in this region an export of nitrogen from wood to bark.

TABLE II.

Mean Excess or Deficit of Ringed Plants as compared with Normal.

(Grm. nitrogen per 6½ inches of stem, on basis of mean Residual Dry Weight.)

	Above the Ring.				Below the Ring.			
	Mean Difference.	Standard Deviation.	Significant Difference.		Mean Difference.	Standard Deviation.	Significant Difference.	
			$P=0.05$.	$P=0.10$.			$P=0.05$.	$P=0.10$.
Bark	+0·00454	0·00214	0·00505	0·00405	−0·00307	0·00384	0·00909	0·00728
Wood	+0·00970	0·00346	0·00817	0·00655	−0·00962	0·00417	0·00986	0·00791
Stem	+0·01424	0·00441	0·01050	0·00836	−0·01269	0·00517	0·01222	0·00979

The results are summarized in Table II, which shows the mean excess or deficit of the Ringed plants as compared with the Normal, together with the statistical data by means of which the changes observed are judged. The increase in nitrogen content of the stem above a ring and the decrease below a ring are both fully significant. It is of interest to note that the response to ringing is much greater in the wood than in the bark. Interchange between wood and bark is clearly rapid. The results are evidently in accordance with the view that there is an upward movement of nitrates through the wood and a downward movement of elaborated nitrogen via the bark from the leaves to the roots.

Something further can now be said concerning the diurnal variations in the total nitrogen of the leaf and the bark. In the leaf the diurnal rise and fall merely indicates that the rate at which nitrates arrive during the day exceeds the rate at which organic nitrogen is exported, and that the reverse obtains at night. The diminution at night in itself shows that there is a withdrawal of organic nitrogen during the night, but there is nothing in the diurnal cycle of the leaf to show whether organic nitrogen is also exported during the day. The diurnal changes in the nitrogen content of the bark and the accumulation of nitrogen that takes place during the day in the stem above the ring show, however, that organic nitrogen is also exported from the leaf during the day. The diurnal changes in the bark suggest also that the rate of export from the leaf may be greater during the day than it is at night. From this it would follow that the rate at which nitrates enter the leaf must be much greater during the day than during the night.

It will be noted that the results obtained in this ringing experiment are the reverse of those obtained by Curtis (4). He found that, while the leaves and the stem tissues *above* a ring do increase slightly in nitrogen content, they increase to a very much *smaller* extent than the corresponding tissues on an unringed plant. It seems probable that the divergence between the two sets of results may be due to the very much longer time interval between ringing and sampling in Curtis's experiments. A short time

interval was adopted in the experiments described in this paper, so that, in ringing, the initial effect of interrupting the continuity of the bark should not be complicated by subsequent morbid changes in the wood. In the ringing experiment just described, the leaves on the Ringed plants did not differ significantly from normal leaves in their moisture content until after 14 hours had elapsed from the operation of ringing. After that time, however, they failed to maintain a normal moisture content. When, as in Curtis's experiments, the minimum time interval is 23 days, the danger of a serious reduction in the transpiration current must be very great.

It may be, of course, that the main seat of synthesis of organic nitrogen compounds is not the same in cotton as in the plants (lilac, privet, peach) with which Curtis worked. In the cotton plant the leaves appear to be the main seat of synthesis; in the lilac, privet, and peach it is possible that the roots may be the main seat of synthesis (cf. Thomas (12)) and that the upward movement of nitrogen is a movement of organic nitrogen via the phloem. Until, however, it is shown that results of the type obtained by Curtis are not due to partial blocking of the wood, this explanation remains unproven.

Closer examination of the results obtained by Curtis in the one experiment designed to eliminate the influence of an altered transpiration current, suggests that even in his plants (privet) the leaves may import considerable quantities of nitrogen compounds (presumably nitrates) via the wood and export organic nitrogen into the stem. In the experiment referred to (Curtis (4), pp. 376–9), analyses were made, 39 days after ringing, of a defoliated region of stem, four internodes long, on twigs of privet. Four pairs of leaves were left above the defoliated region. A ring of bark was removed just above the defoliated region in one group of plants (High Ring group), just below the defoliated region in a second group of plants (Low Ring group), and both above and below the defoliated region in a third group (Two Ring group). It was assumed, since all stems were ringed and had the same number of leaves above a ring, that transpiration would be approximately the same in all groups. On analysis the total nitrogen contents, per twig, of the defoliated region were found to be: High Ring group, 12·11 mg.; Low Ring group, 10·75 mg.; Two Ring group, 7·45 mg. Taking the Two Ring group as a base-line, which seems fair, we note that in the first group (High Ring) there is a gain of 4·66 mg. and in the second group (Low Ring) a gain of 3·30 mg. The gain in the Low Ring group is 70·8 per cent. of that in the High Ring group, in spite of the fact that in the latter group the defoliated region is in contact *via the bark* with the whole of the foliage of the plant except the eight leaves at the apex; whereas in the former group the defoliated region is in contact *via the bark* only with the eight leaves at the top of the plant. These eight leaves, moreover, are drawing their nitrate supply *via the wood past the region of*

a ring, whereas the nitrate supply to the rest of the foliage is in no way impeded. In spite of this disability, these eight leaves supply to the defoliated region 70 per cent. as much nitrogen as do all the leaves on the rest of the plant. Thus this experiment, far from supporting the conclusions which Curtis draws from his other experiments, emphasizes the ability of the leaves to import from the wood nitrogen compounds which move upwards in that tissue, and from these to synthesize organic nitrogen compounds which are re-exported via the bark to other parts of the plant.

3. *The Effect of separating Bark and Wood* (Experiment 3 (7)).

Procedure. In this experiment there were three groups of plants, treated in the manner shown in Fig. 6. The plants were marked with wool at two places on the stem. One mark was situated where the lowest fruiting-branch joined the main axis, the second was seven inches below it. The stem below the lowest fruiting-branch had been cleared of all leaves and branches some days previously. About an hour before the time of the first collection all the plants were ringed at the lower mark. The width of the ring was half an inch. Four vertical cuts, each seven inches long, were then made upwards from this ring. The width of the vertical cut is greatly exaggerated in the figure. In the first or Normal group the only additional treatment was to cover the stem with paraffined paper. In the second or Flap group the bark was carefully levered up from the wood to form four flaps, only two of which are shown in the figure. These flaps were still continuous with the bark above. The surface of the wood was covered with vaseline, the flaps replaced in their normal position and held with string. Paraffined paper was then placed around the stem. The third or Cut Flap group was treated in an exactly similar manner, with the addition that the flaps of bark were severed from the bark above. The flaps of bark in this group were thus completely isolated. The treatments accorded the three groups were therefore as follows:

1. Normal group. A ring of bark removed seven inches below the first fruiting-branch, and four vertical cuts made through the bark extending from the ring to the first fruiting-branch.
2. Flap group. A ring of bark removed seven inches below the first fruiting-branch. The bark between this ring and the first fruiting-branch separated from the wood to form four flaps; the wood covered with vaseline and the flaps of bark replaced in their normal position.
3. Cut Flap group. As Flap group, but the flaps were severed from the bark at the top.

On being brought into the laboratory, the seven-inch pieces of stem were trimmed to six and a half inches, rejecting the half-inch of stem below the

first fruiting-branch, and the bark and wood were weighed. There were two samples per collection, each of nine plants.

Results. The weights of nitrogen in the wood and bark respectively of the six and a half inches of stem sampled are shown in Fig. 7. The

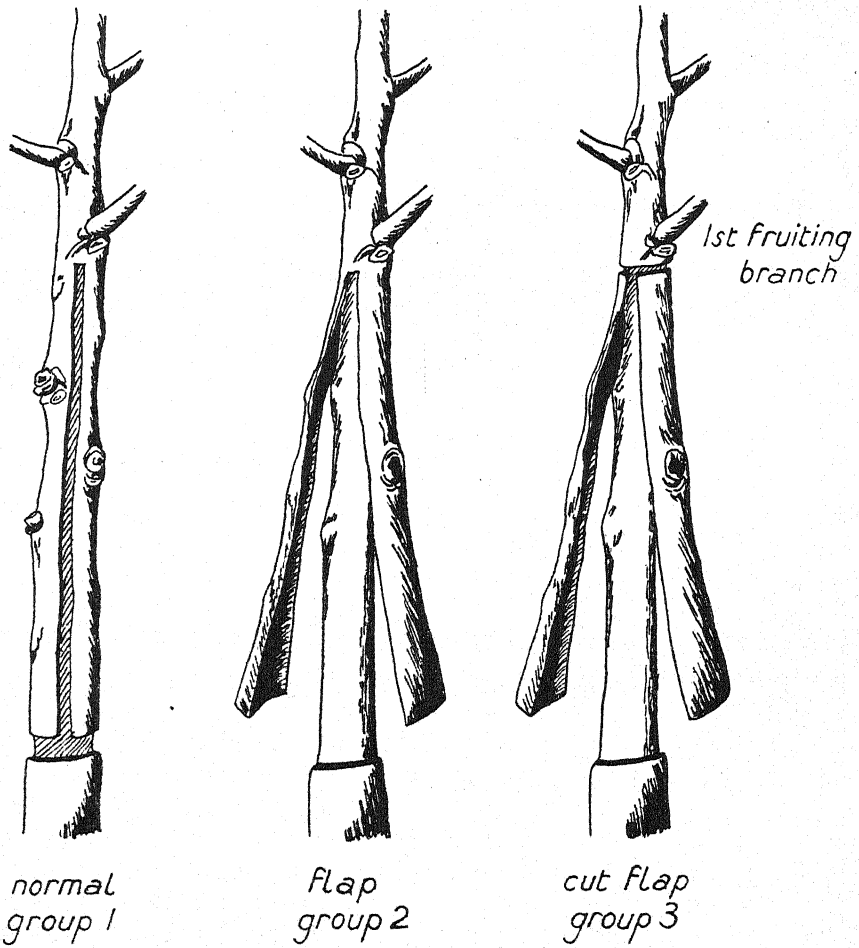


FIG. 6. Diagrams of the three groups of stems with 'flaps'.

results are expressed only on the residual dry weight basis. The fresh weight basis has not been employed, as the moisture conditions of the bark were appreciably affected by the treatment. It will be observed that the amount of nitrogen in the bark of the Normal and Flap groups was much greater than in that of the Cut Flap group. The mean nitrogen content of Flap and Normal groups is almost identical. The Cut Flap group, on the other hand, has a mean nitrogen content significantly less than that of either the Normal or the Flap groups. It is clear, therefore, that nitrogen

has not only entered the bark that was in contact with the wood, but has passed also into the isolated flaps of bark. Contact between wood and bark is therefore unnecessary for the normal transport of organic nitrogen from the leaves towards the roots. There is no indication whether there is a movement of nitric nitrogen from wood to bark via the medullary rays.

In the wood the amount of nitrogen was much greater in the Normal than in either of the Flap groups; the difference presumably being due to an ingress of nitrogen from the bark. As the wood of neither the Flap nor the Cut Flap group was in contact with the bark, it is not clear why the Flap group should show a consistently greater amount of nitrogen than the Cut Flap group. The difference is not, however, statistically significant, whereas the difference between the Normal group and either of the Flap groups is fully significant. The results are summed up in Table III, which gives the mean values for the three collections in each group, together with the necessary statistical data.

TABLE III.

Grm. Nitrogen in Bark and Wood of the six and a half inch Section of Stem below the First Fruiting Branch.

	Normal.	Flap.	Cut-Flap.	Standard Deviation.	Significant Difference.	
					$P = 0{\cdot}05$.	$P = 0{\cdot}10$.
Bark	0·14870	0·14863	0·13747	0·00336	0·01059	0·00861
Wood	0·14327	0·12887	0·12357	0·00231	0·00729	0·00592

The interpretation of the results seems clear. In the Normal group nitrogen moved down into the bark and thence to the wood, so that both bark and wood contain more nitrogen than the Cut Flap group. In the Flap group nitrogen moved into the bark only. Using the Cut Flap group as a base-line, we find that in eighteen hours (mean time for the three collections) 0·03093 grm. nitrogen moved down into the bark below the first fruiting-branch on the Normal plant, and that 63·8 per cent. of this nitrogen (0·0197 grm.) moved on into the wood. In the Flap group 0·01117 grm. of nitrogen moved down into the isolated bark. It is interesting to note that, as in the ringing experiment, the response to treatment is greater in the wood. The rate of movement involved will be considered in a subsequent section.

4. *The Effect upon the Rate of Transport of a Constriction in the Path of Transport* (Strip Width Experiment (8)).

Procedure. In this experiment there were four groups of plants, all of which had approximately two feet of bare stem between the first fruiting-branch and the ground. The operation of ringing and removing the different

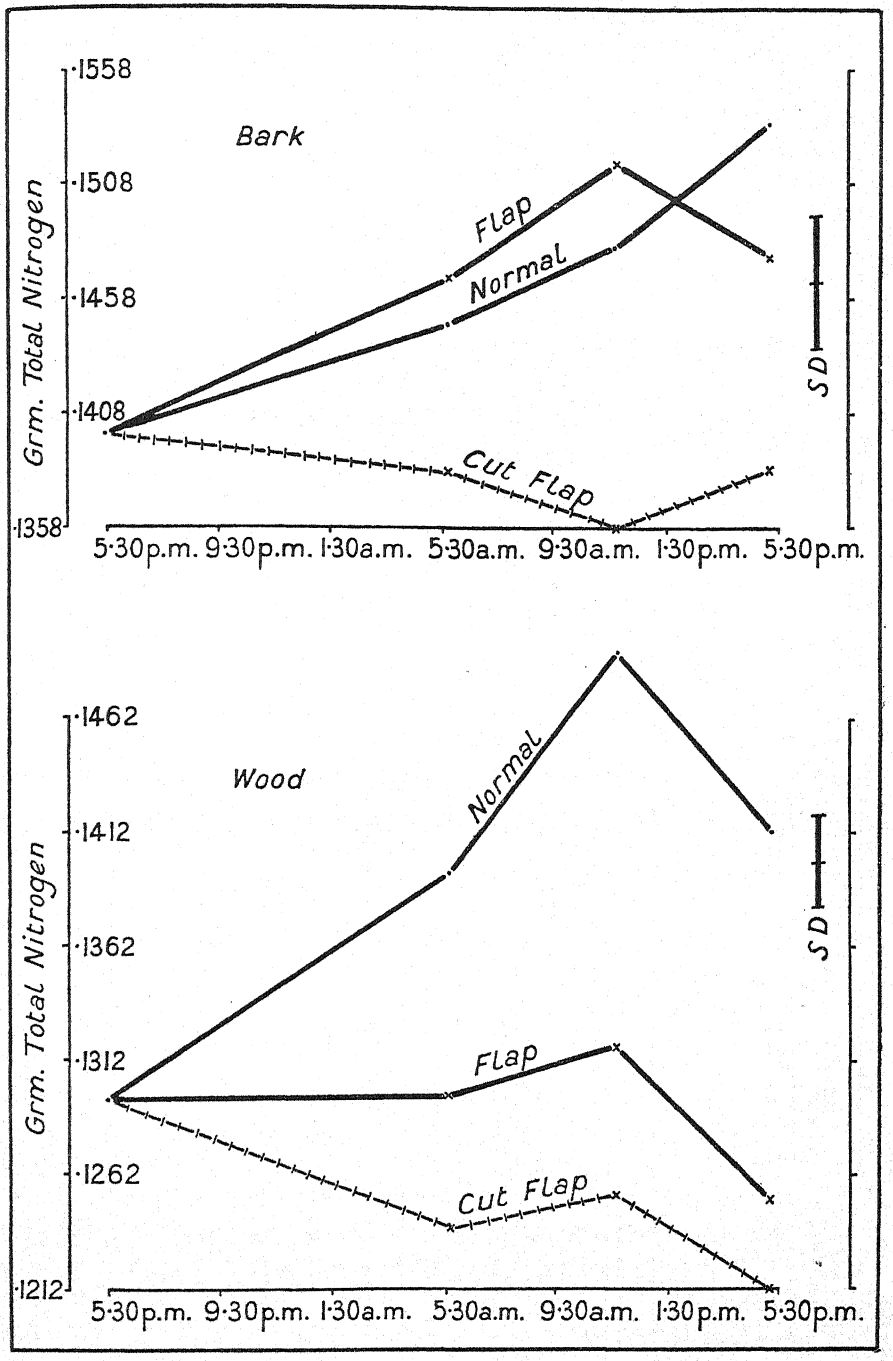


FIG. 7. Weight of nitrogen in bark (upper) and wood (lower) from 6½ inches of stem. S.D. = Standard deviation due to sampling.

fractions of bark was done between 6 a.m. and 7 a.m. on the 23rd of November. A quarter-inch ring of bark near the ground-level was removed from the plants of all four groups. In the first group, which will be

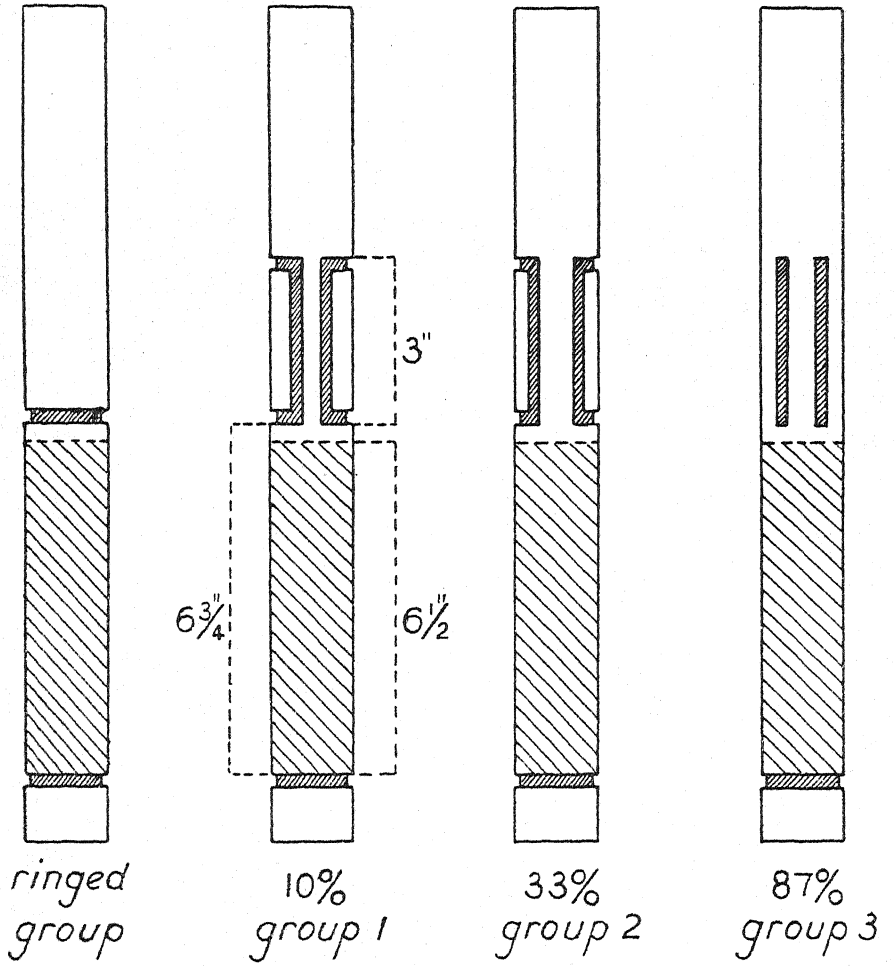


Fig. 8. Diagrams of the four groups of ringed stems.

referred to as the Ringed group, another ring was made seven inches above it. In the second group a strip of bark, in width approximately 10 per cent. of the whole circumference, was left bridging the gap above and below the constriction (see Fig. 8). The length of this bridge was three inches. The bridge was made by making two partial rings connected with each other by two vertical cuts. The third group was similar except that the bridge of bark was 33 per cent. of the total circumference. In the fourth group there were only two vertical cuts, each three inches long; the bark

remaining constituted 87 per cent. of the total circumference. Though the ringing and the removal of fractions of bark was done at dawn on the 23rd of November, the first collection was not made till 6.40 a.m. the following morning. For each collection there were three samples of seven plants. The weight of nitrogen in the six and a half inch section of stem below the constriction was determined in the usual way. The region sampled is marked with diagonal lines in the diagram.

Results. The weight of total nitrogen in the six and a half inches of stem (bark and wood) below the region of the constriction is shown in Table IV. The results are calculated on the residual dry weight basis. It will be noticed that the plants of the Ringed group contain on the average a smaller amount of nitrogen than the other groups.

TABLE IV.

Grm. Nitrogen per six and a half inches of Stem.

Time.	Hours from Start.	Ringed.	87 per cent.	33 per cent.	10 per cent.
6.40 a.m.	24·166	0·19133	0·20191		
7.45 a.m.	25·25			0·19200	0·18910
5.0 p.m.	34·50	0·18710	0·20505		
6.9 p.m.	35·50			0·20657	0·20028

Standard deviation = 0·003606.

The weight of nitrogen transported has been calculated by deducting the weight of nitrogen in the Ringed from the other groups. As the hours of collection were not identical, the excess over the Ringed group has been calculated at the mid-points in time between the two morning and the two evening collections. The weight of nitrogen transported is shown in Table V. It will be seen that the amount of nitrogen transported falls off as the width of the strip narrows. The amount moved in the *87 per cent. group* is significantly greater than that moved in the *10 per cent. group*. The *33 per cent. group* is intermediate, but does not, however, differ significantly from either of the others.

TABLE V.

Grm. Nitrogen Transported. Strip Width Experiment.

Hours from Start.	87 per cent. Group.	33 per cent. Group.	10 per cent. Group.
24·708	0·01097	0·00012	0·00260
35·00	0·01831	0·01907	0·01285
Mean 29·85	0·01464	0·00956	0·00513

Standard deviation of mean = 0·00361.
Significant difference: ($P = 0·05$) = 0·00764; ($P = 0·10$), 0·00630.

The reduction in the rate of transport as the strip is narrowed is not

proportional to the reduction in cross-sectional area of the strip, so that the rate across unit area of the strip increases as the strip narrows. The rates are given in Table VI, the mean value for the two collections being used.

TABLE VI.

Rate of Transport.

Group.	Area of Sieve-tubes in Strip sq. cm.	Rate of Transport of Nitrogen. Grm. per sq. cm. per hour. (*a*) Expressed as grm. Nitrogen.	(*b*) Expressed as grm. Asparagine.	Rate of Transport of Carbohydrate. Grm. per 1 sq. cm. per hour.
87 per cent.	0·104	0·004715	0·02527	0·2476
33 per cent.	0·0394	0·008129	0·04356	0·5911
10 per cent.	0·0111	0·015480	0·08296	1·4913

It was pointed out, in discussing the results for carbohydrates (8), that the results were in harmony with the view that movement of carbohydrate was analogous to movement by diffusion, since the constriction of one part of a diffusion channel would have the effect of steepening the gradient across the constriction, and in consequence would increase the rate of transport across unit cross-sectional area in the constricted region. In the case of the carbohydrates we were able to show that the gradients in sugar concentration were, in fact, increased by approximately the amount required to account for the observed increase in rate. In the case of nitrogen we have no information as to gradients, but, so far as they go, the results suggest that movement of organic nitrogen may, like movement of sugars, be analogous to movement by diffusion. The absolute rates indicated are, as in the case of the sugars, far in excess of the rates which would be given by purely physical diffusion. It is only the pattern of movement which is similar in both cases.

5. *Rates of Movement.*

Certain general features of the results of the experiments may now be considered. To take first the question of the absolute rates of movement. Table VII summarizes the data available from the present set of experiments. The data are calculated from the means of all collections subsequent to the initial collection.

In the ringing experiment the difference in nitrogen content between the stem of the Normal plant below the tape and the stem of the Ringed plant below the ring is taken as a measure of the amount moved in the Normal plant. In the case of the strip width experiment the sieve-tube area is taken as the average area between the mid-points of the two regions of stem sampled above and below the constriction. This was done in the analysis of the carbohydrate data in order to make comparison between

the rate of movement and the gradient observed between upper and lower regions of stem. The same procedure has been followed here in order to compare the nitrogen rates with the carbohydrate rates.

TABLE VII.

Rate of Downward Movement of Nitrogen.

Experiment.	Mean Time, Hours.	Mean Amount Moved.	Cross-sectional Area of Sieve-tubes sq. cm.	Grm. per sq. cm. of Sieve-tubes per hour.	
				Nitrogen.	Carbohydrate.
Ringing Expt.	12·68	0·01269	0·187	0·005352	0·2356
Flap Expt.:					
(1) Normal Stem	18·00	0·03093	0·1943	0·008843	0·1510
(2) Bark Flap	18·00	0·01117	0·1943	0·003211	0·0794
Strip Width Expt.:			0·1135		
(1) 87 per cent.	29·85	0·01464	0·0687	0·004320	0·2267
(2) 33 per cent.	29·85	0·00956	0·0258	0·004660	0·3390
(3) 10 per cent.	29·85	0·00513		0·006670	0·6423
			Mean	0·005509	0·2790

Mean rate of nitrogen transport calculated as grm. asparagine = 0·02953.

It will be seen that although, in the strip width experiment, the rate of transport of nitrogen increases, along with that of carbohydrate, as the strip is narrowed, yet the rate of nitrogen transport does not increase as rapidly as the rate of carbohydrate transport. If the mechanism of nitrogen transport is similar to that of carbohydrate transport, i.e. if direction and rate of movement are determined by gradients of diffusible substances in the channels of transport, it would appear that the nitrogen gradient across the constricted region has not increased as much as the sugar gradient: a result which suggests that fluctuations in concentration of diffusible nitrogen compounds may readily be damped by conversion of the excess to a less diffusible form.[1] Whether this is the case or not, it will be seen that the rate of nitrogen transport varies in the series of experiments much less than the rate of carbohydrate transport. The standard deviation for nitrogen is 36·2 per cent. of the mean rate, and for carbohydrates 71·1 per cent. of the mean rate. It may be noticed also that there is no consistent relation in the different experiments between the rate of carbohydrate transport and that of nitrogen transport; the correlation coefficient + 0·239 is quite insignificant.

The great disparity between the rates of nitrogen transport and those of carbohydrate transport given in Table VII is, of course, in part due to the fact that carbohydrates are expressed in terms of a compound, glucose, whereas nitrogen compounds are expressed in terms of the element, nitrogen. If the rate of nitrogen transport is expressed, for instance, in terms of

[1] We noted in the earlier papers that such damping hardly occurs at all in the case of sugars.

asparagine, the average rate of transport is as much as 14·74 per cent. of the rate for sugars. The average weight of *nitrogen* moving downwards is, however, only 2·75 per cent. of the amount of sugars moving downwards.

As the nitrogen content of the lower region of the stem in the cotton plants considered here, is roughly 2 per cent. of the total dry weight, it would seem that on the average, in the downward movement of organic substances in these plants, nitrogen and carbohydrate are present in about the proportions required for growth. Now as the whole of the carbohydrate required for new growth is coming from above, it would seem that the whole of the combined nitrogen may also be coming from above. A more accurate comparison between the rate of downward movement of nitrogen and the rate of addition of nitrogen in new growth is, however, required to test this possibility.

6. *Response of Bark and of Wood*

An additional point of interest in comparing the results for nitrogen and for carbohydrate lies in the very different response shown by bark and wood in the two cases. In both cases the bark is the main channel of longitudinal transport, but there is also temporary storage of material in the wood. For the group of experiments considered in this paper, we find that rather more than half the nitrogen (50·7 per cent.) present in a given length of stem is in the wood. In the case of sugars, on the other hand, only 32·1 per cent. of the total is in the wood, though the wood contains 55·7 per cent. of the total carbohydrates. Now, although bark and wood contain approximately equal amounts of nitrogen, the response shown to those changes in nitrogen supply or demand caused by ringing or partial ringing is very much greater in the wood. The average change in the wood—amount of nitrogen gained or lost during an experiment—amounts to 8·48 per cent. of the mean amount present: in the bark the average change is only 4·74 per cent. of the mean amount present. In the case of sugars, although less are present in the wood, the percentage change is about equal in wood and bark (40·5 per cent. and 39·6 per cent. respectively), while for total carbohydrates, although less are present in the bark, the percentage change there is greater (28·8 per cent. as against 13·3 per cent. for the wood). It seems clear that both the proportion and the absolute amount of labile nitrogen must be greater in the wood than in the bark, whereas both the proportion and the absolute amount of labile carbohydrate is greater in the bark than in the wood. While the interest of this fact is obvious, its significance is not as yet clear.

One further point of general interest may be considered. In the work on carbohydrates we found that in a defoliated region of stem between two rings the carbohydrates may fall quite markedly as a result, presumably, of

respiration. In twenty-four hours the average loss was, in the bark 19·5 per cent., and in the wood 9·9 per cent. There is, in the case of nitrogen also, some loss from a region of stem between two rings, but it is very much smaller. On the experiments considered here it amounts, for the bark, to 1·94 per cent., and for the wood, to 3·46 per cent. The cause of this small loss is not clear, but it is interesting to note that it is greater in the wood.[1]

Discussion.

The data presented are in accord with the view that the bulk of the mineral nitrogen absorbed by the roots is carried in the transpiration current to the leaves, where it is elaborated to amino-acids, &c., and then exported through the sieve-tubes back to the roots, &c. In the first place, the movement of inorganic nitrogen into the leaves is not appreciably affected by the removal of a ring of bark. Secondly, the total nitrogen of the leaf increases by day and diminishes during the night. This reduction must indicate that nitrogen is exported from the leaf at night. Inasmuch as nitrogen was transported through isolated flaps of bark, and as, moreover, the nitrogen content of the bark is shown in the ringing experiment to increase by day and diminish by night, it would appear that not only is organic nitrogen exported from the leaf both by day and night, but the rate of transport is greater by day than it is by night. As a corollary to this, it would follow that the rate at which nitrates enter the leaf is much greater during the day than at night.

If the absorption of nitrates by the roots is independent of the rate of transpiration and continues at an approximately uniform rate throughout the day-night period, there ought to be an accumulation of nitrogen in the roots during the night while transpiration rates are low. The removal of nitrates by root-pressure, if it occurs at all, must be very small. Information on the diurnal variations of the nitrogen content of the roots is, however, wholly lacking.

It is difficult to see why nitrates should accumulate in the leaf by night, as Chibnall (2) has shown, if their synthesis is a purely chemosynthetic process, for though there is a reduction at night in sugar concentration, there is probably a much greater reduction in the rate at which nitrates enter the leaf.

The machinery of nitrate transport seems tolerably clear, for if it be admitted that nitrates travel in wood, it ought to follow that they move with the transpiration current, and the factors that affect the rate of

[1] It will be noted that this rate of loss is very much less than the rate of loss of nitrogen from the stem below the ring in the first ringing experiment. There the rate of loss (calculated for twenty-four hours) was, bark 7·37 per cent., wood 16·15 per cent. This makes it quite clear that the loss below the ring in the ringing experiment was due to downward transport to the roots.

transpiration must affect their rate of transport from root to leaf. The distribution to the leaf of the bulk of nitrates absorbed by the root seems a necessary consequence of their movement in the transpiration stream. It is not altogether certain whether nitrates in small amounts may not also move in the sieve-tubes, for appreciable quantities have been found in the bark tissues.

The factors responsible for the movement of organic nitrogen in the sieve-tubes are more complex. The effect of constricting the channel of transport suggests that diffusion is a factor in the process of transport. As the rate of movement is greatly in excess of that due to diffusion, it appears probable that, as in the case of sugar transport, there is some agency at work accelerating diffusion in the sieve-tube. It will be clear that a great deal more work is needed before anything in the nature of an adequate theory of nitrogen transport is forthcoming.

Summary.

1. The nitrogen content of the leaf increases by day and diminishes by night.

2. The nitrogen content of the bark, just below the foliage region, of plants bearing bolls shows no appreciable diurnal variation.

3. In plants without bolls the nitrogen content of the bark increases during the day and diminishes by night.

4. Removal of a ring of bark causes an accumulation of nitrogen in bark and wood above the ring, and a decrease in bark and wood below the ring. It does not prevent movement of inorganic nitrogen into the leaf.

5. Downward movement of nitrogen will take place through isolated flaps of bark.

6. Isolation of the wood from the bark prevents the accumulation of nitrogen in the wood above a ring.

7. Removal of varying fractions of the bark brings about a decrease in the total amount of nitrogen transported, but an increase in the rate of transport, per unit cross-sectional area, over the constricted region.

8. The average weight of nitrogen compounds, expressed as asparagine, moving down the stem was 14·75 per cent. of the average weight of carbohydrates moving downwards.

9. On the average, the absolute amount of nitrogen in the wood of the lower part of the stem is about equal to that in the bark, but the variation in the wood in response to variations in supply or demand is about twice that in the bark.

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The Biological Decomposition of Pectin.

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With four Figures in the Text.

INTRODUCTION.

THE decomposition of the pectic substances is a subject of great interest to the plant pathologist, since these substances form an invariable constituent of the cell-walls of unlignified tissue, and in particular, of fruits. Our knowledge of these processes is, however, fragmentary. Misconceptions as to the real nature of pectin have hindered progress in this direction, but the work of Nanji, Paton, and Ling (1), and that of Norris and Schryver (2) having thrown much light on its constitution, the biological aspect can now be studied with hope of progress.

Apart from the standpoint of plant pathology, an understanding of the microbiological degradation of these substances is of commercial importance in connexion with the rotting of stored fruit, potatoes, &c., the retting of fibre plants, such as flax, and hemp, and the nature and course of the breakdown in the soil, and the manure heap.

Briefly considering each in turn; the invasion of a plant tissue, whether by bacteria, or by fungi, involves passage of the colony or mycelium either along the middle lamella between the cells, or else direct penetration of the cell-walls. The solution of the middle lamella is probably the most widespread action in plant diseases, although penetration may be aided by splitting of the tissue owing to mass action by rapid multiplication of a bacterial colony, or ramification of a mycelium, in a closed space.

Cooley (3) maintains that the hyphae of *Sclerotinea cinerea* in plum tissue are mainly intracellular, stating that he was unable to show the presence of any enzyme capable of dissolving the middle lamella, and that he could find no trace of any softening in advance of the hyphae. Valleau (4) in investigations with the same fungus, came to practically opposite conclusions. He described an enzyme, termed by him 'pectinase', which was responsible for the destruction of the middle lamella. Softening in advance of the mycelial strands was stated by him to have been observed. This softening he ascribed to the action of oxalic acid, presumed by him to be

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excreted by the penetrating hyphae. Hawkins (5) was of the opinion that none of the pectic material is assimilated by the fungus, since he found no diminution in the pentosan content of peaches rotted by it.

Very similar results have been described in the case of bacterial action. Potter (6) studied the bacterial rot of turnips, and stated that the rot was due to the solvent action of oxalic acid produced by the bacteria. Erwin F. Smith (7) contended that oxalic acid alone had no solvent action on the middle lamella of turnips, but that ammonium oxalate would dissolve it. As bacteria are stated to evolve ammonia during growth, he considered it not unlikely that it is ammonium oxalate which in some cases dissolves the middle lamella. More recently, many workers have stated that a specific enzyme is responsible for the rotting observed.

The work of Willaman (8), (9), (10), (11), on the growth of *Sclerotinea* on certain tissues, provides additional evidence for this last statement. He estimated the destruction of pectin by the action of the fungus *in vitro*, by performing furfural estimations both before inoculation and after several weeks growth, and showed that pectin was assimilated by it in the absence of easily assimilable sugars. Furfural determinations are, of course, not a direct method of estimation of pectin, since they only indicate the number of furfural yielding groups, which may be unchanged pectin, but which, also, may be pectin degradation products, uronic acids, pentosans, and pentoses. Further, the sample of pectin employed by this worker for the preparation of his culture medium, prepared by alcoholic precipitation of prune juice, must have been of low purity since the furfural yield was only of the order of 15 per cent., as opposed to the theoretical which approaches 20 per cent. Willaman concludes that when this fungus invades a tissue, it follows the line of the middle lamella by dissolving out the latter by means of the enzyme 'pectosinase'. It then reprecipitates the dissolved pectin as calcium pectate. The latter being a hydrophyllic gel is presumed to maintain the firmness of the fruit after rotting, which is a characteristic of fruit rotted by *Sclerotinea*. This highly imbibing gel is also supposed to be of use to the fungus in maintaining a supply of water. It is to be noted that he assumes the middle lamella to be composed of protopectin, an assumption not generally recognized; and even though he shows that pectin is assimilated by the fungus *in vitro*, merely assumes that it is 'reprecipitated as calcium pectate' *in vivo*. This paper is of importance since it contains the first reference to an attempt to follow quantitatively *in vitro*, the decomposition of pectin observed to take place *in vivo*. In the two later papers, Willaman (9), (10) gives a remarkable list of estimations recording the changes of the composition of fruit brought about by the rotting effect of this fungus.

Turning again to bacterial diseases, considerably more evidence is available to show that the solution of the middle lamella is brought about

by enzyme action. Spiekerman (12) isolated an enzyme from the expressed juice of the carrot attacked by a bacterial soft rot. He showed that the dissolving power which this juice possesses is destroyed by boiling. He inoculated this bacterium into an artificial medium of calcium pectate, containing some nitrogenous material, and found that the pectate slowly disappeared, the end point being reached when pentose, and probably also hexose, is still present in the solution. Further degradation of these substances does not appear to take place outside the bacterial cell. The enzyme, according to this author, makes possible a rapid penetration to the more remote tissues, of the 'poison' secreted by the bacteria; by the resulting death of the protoplasm the cell-sap, rich in nutrient substances, is made accessible to the bacteria, thereby ensuring rapid growth and multiplication. In addition, the products of the degradation of the middle lamella are an immediate source of nutritive material, and the calcium carbonate formed from the pectate, combines with the acids produced by the bacteria, which might otherwise restrict their development.

Jones (13) studied the enzyme produced by *Bacillus carotovorus*, a very active bacillus rotting only parenchymatous tissues. Careful consideration of its reactions, and those of other plant pathogenic bacteria, cause the author to conclude that cytolytic enzymes secreted by the bacteria are not simple, but fall into at least two major groups, one of which attack the pectic, and the other the cellulosic constituents of the cell-wall. He states that the enzyme of *B. carotovorus* and of the allied soft-rot bacteria act upon the pectic compounds only.

Dealing now with industrial processes involving the micro-biological decomposition of pectin, the chief example is the retting of fibre plants, such as flax and hemp for the isolation of the fibres, or hard bast, which lie embedded in the parenchymatous tissue of the cortex. By the solution of the middle lamella of the phloem and cortical tissue the bundles become separated from the woody matter, which is removed later, after drying, in the process of scutching. Eyre and Nodder (14) have made a detailed study of the course of the operations in the retting tank, without, however, considering in detail the decomposition of the pectic substances. They divide the ret into two stages, the first a physical, and the second a biological stage. In the first, after the immersion of the dried straw in water, swelling takes place, and many soluble substances pass into solution. In the first phase of the biological stage, active fermentation of these soluble substances takes place by means of many aërobic bacteria, adventitiously present, thereby creating anaërobic conditions in the water, and paving the way for the anaërobic pectin fermentation. In the second part of the biological phase the water-soluble pectic substance appears to be fermented away. This stage is not entered upon until all the readily fermentible substances have been removed. During the third, and principal part of the

biological stage, the main fermentation of the pectic substance soluble in ammonium oxalate takes place, and rapid loosening of the fibre-bundles occurs. Finally, if the ret is prolonged a fourth stage is reached, during which the pectin of the bundles is attacked, and their tensile strength adversely affected. Many bacteria play a part in the retting process, the chief one being stated to be *Plectridium pectinovorum*, an anaërobic organism.

The decomposition of the pectic substances in the soil, and the probable production from them of simple carbohydrate material is of great significance in relation to the nitrogen cycle, and also not improbably, to the formation of humic material. Very little is known of this degradation in the soil, but there must be a restricted class of soil micro-organisms capable of bringing about this change. The production of synthetic farmyard manure by fermentation of vegetable material, under certain regulated conditions, must also involve similar changes in the pectic substances.

Experimental.

With the object of following *in vitro* the 'pectic relationships' of certain of the organisms involved in some of the processes mentioned above, a method was devised which gives an indication of the rate of decomposition, both of pectin, and of its degradation products.

Growth was allowed to take place on various artificial pectin media, containing a concentration of pectin in some cases up to more than 2 per cent. Of the concentrations tested, 1 per cent. would seem to be the most suitable. The pectin employed was a pure powdered product, prepared from lemons according to the method of Wilson (15). Some source of nitrogen has to be added to the medium. Preliminary experiments with *B. carotovorus* showing that 1 per cent. peptone is the most suitable. Inorganic salts are added to the extent of 0·2 per cent. potassium dihydrogen phosphate, and 0·1 per cent. magnesium sulphate. When the medium has been prepared, about 20 grm. of fine precipitated chalk per litre are added, and stirred in with a mechanical stirrer, so that it is quite evenly distributed through the solution. Into each of a series of tubes is measured out 10 c.c. of this, and the tubes plugged in the usual way. Some difficulty is experienced in dealing with media containing a concentration of pectin more than 1 per cent., owing to the high viscosity. However, if a graduated pipette with a fairly large jet be employed, and the quantity measured by difference, the error is not serious. Sterilization is carried out by steaming for twenty minutes on each of three successive days, the tubes being incubated in the interim. As a rule the chalk is not completely deposited by this method of sterilization. Pressure sterilization in an autoclave is to be avoided since degradation of the pectin undoubtedly occurs. The chalk

serves to keep constant the hydrogen-ion concentration of the medium during the growth of the organism, by combining with the acids formed on degradation.

To follow the destruction of the pectin, and its degradation products, the following determinations are carried out from time to time:

(1) Furfural yield.
(2) Calcium pectate yield.

In each case a tube is taken and washed out with hydrochloric acid. For furfural determinations 100 c.c. of 12 per cent. acid is employed, and the furfural produced on distillation under the usual standard conditions, precipitated by phloroglucinol. The phloroglucide is filtered off on a sinter-glass Gooch funnel, extracted with boiling absolute alcohol, dried and weighed. For the calcium pectate estimations, the tubes are washed out with about 50 c.c. of 10 per cent. hydrochloric acid, and the pectin precipitated by the addition of about three and a half volumes of 95 per cent. alcohol. After standing for several hours the precipitate is filtered off on a fluted filter-paper, and washed with acid alcohol. The filter-paper with the precipitate is then placed in a beaker, and the precipitate dissolved by boiling with about 50 c.c. distilled water. The solution is filtered off and the paper boiled again, this time with dilute ammonia, so that any pectic acid which may happen to be present, may not be lost. Finally, the filter-paper is triturated with a further small quantity of water, boiled and filtered. The combined filtrates and washings are treated with 100 c.c. N/10 sodium hydroxide, and allowed to stand overnight. 50 c.c. of N. acetic acid, and an equal volume of N. calcium chloride are added, and after boiling for at least five minutes, the solution is filtered hot through a tared Swedish paper. After prolonged washing with hot water until the washings show no trace of chloride, the paper is dried and weighed.

It is clear that the yield of furfural is due both to the unchanged pectin, and to pectin degradation products, formed owing to the activity of the organism. From the yield of calcium pectate may be calculated the furfural due to unchanged pectin, the difference being that due to pectin degradation products. If it is observed that the difference figure is very small, and pectin is still being broken down, then it can be assumed that the organism in question makes use of the degradation products of pectin formed by it, more readily than it utilizes the pectin itself. If on the other hand, the furfural due to degradation products is high, and the pectin is stilll broken down, the conclusion to be drawn is that there is present normally in the organism an enzyme which will decompose pectin, and that the organism is of the true pectin fermenting type.

As an example of the use of this method the rate of decomposition of pectin produced by the following plant pathogenic bacteria was studied:

Bacillus carotovorus.
Bacillus atrosepticus.
Bacillus solanisaprus.

Later, the very common hay bacillus, *B. subtilis*, which has been found by Hauman (16), and others in retting liquors, was examined to determine

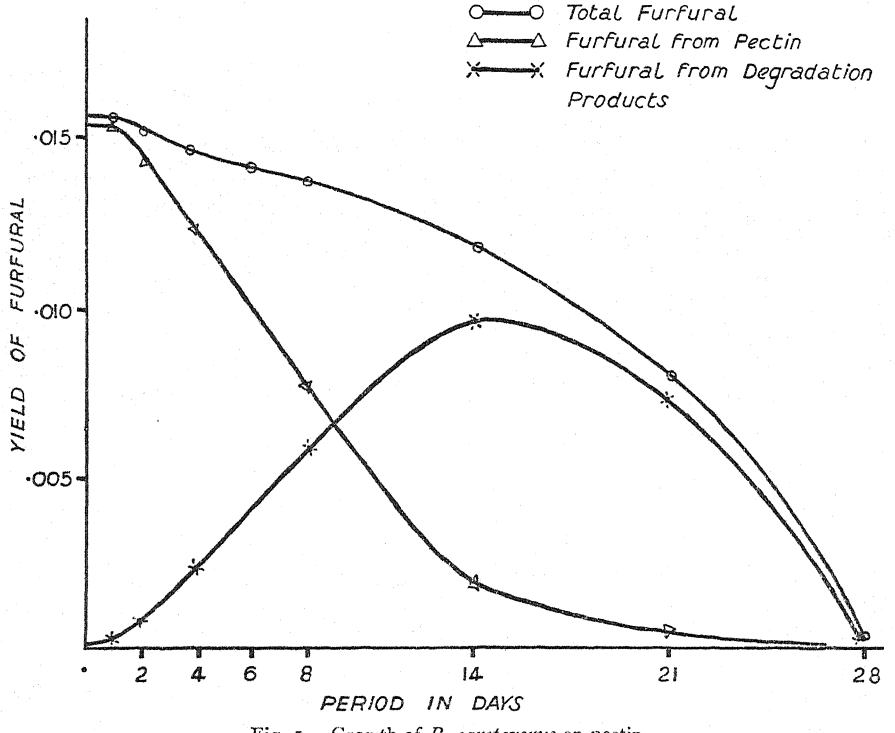


Fig. 1. Growth of *B. carotovorus* on pectin.

whether or no it is a true pectin fermenting organism, that is to say, whether or no it will ferment pectin in presence of other simpler degradation products.

The bacteria in question were sub-cultured on standard potato agar previous to inoculation. An even loopful of the culture was in each case rubbed off against the side in the pectin medium. The tubes were then incubated at 30° C.

I. Action of *B. carotovorus* on pectin.

It is clear then that *B. carotovorus* breaks down pectin actively, a result quite expected since this particular organism produces a quick and complete rot of carrots, turnips, potatoes, &c. There can be no doubt that it secretes normally a pectin decomposing enzyme, since the pectin disappears

at a much faster rate than its degradation products are utilized for food. Degradation products accumulated to the extent of 0·2 per cent. calculated as pentose.

Results expressed on 10 cc. medium.

Time in Days.	Total Furfural.	Calcium Pectate Yield.	Furfural from Pectin.	Furfural from Degradation Products.	Degradation Products Calculated as Pentose.
Control	0·0155	0·08	0·0153	0·0002	0·0004
1	0·0155	0·08	0·0153	0·0002	0·0004
2	0·0151	0·74	0·0142	0·0009	0·0017
4	0·0146	0·64	0·0125	0·0023	0·0045
6	0·0142	—	—	—	—
8	0·0138	0·41	0·0079	0·0059	0·0115
14	0·0118	0·10	0·0019	0·0099	0·0192
21	0·0081	0·003	0·0006	0·0075	0·0146
28	0·0005	trace	—	0·0005	0·0009

These results are indicated graphically in Fig. 1.

II. Action of *B. atrosepticus* on pectin.

A more concentrated solution of pectin was employed in this series. The results are expressed per 100 c.c. of medium.

Time in Days.	Total Furfural.	Calcium Pectate Yield.	Furfural from Pectin.	Furfural from Degradation Products.	Degradation Product Calculated as Pentose.
Control	0·416	2·06	0·395	0·021	0·041
2	0·410	2·03	0·389	0·021	0·041
5	0·405	1·98	0·379	0·026	0·051
8	0·401	1·95	0·374	0·027	0·052
15	0·363	1·48	0·284	0·079	0·154
22	0·317	1·00	0·192	0·125	0·243
28	0·236	—	—	—	—

The results appear graphically in Fig. 2. It will be seen that this organism is even more rapid in decomposing pectin than the previous example. Furfural yielding products other than pectin increased steadily in the cultures. The graph is rapidly assuming the characteristics of Fig. 1. It was noted that at the end of the first ten days a flocculate precipitate appeared, and an attempt was made to isolate this substance. On rendering the culture slightly alkaline the precipitate went into solution. The chalk and other particles were then filtered off, and the substance again precipitated by rendering the solution neutral. If taken over to the acid side it goes again into solution. Several large fermentations were carried out on quantities up to 1 litre, but it was not found possible to isolate sufficient of the material to enable a full investigation of its composition to be undertaken. When this can be achieved, valuable light may be thrown on the nature and route of the biological decomposition of pectin.

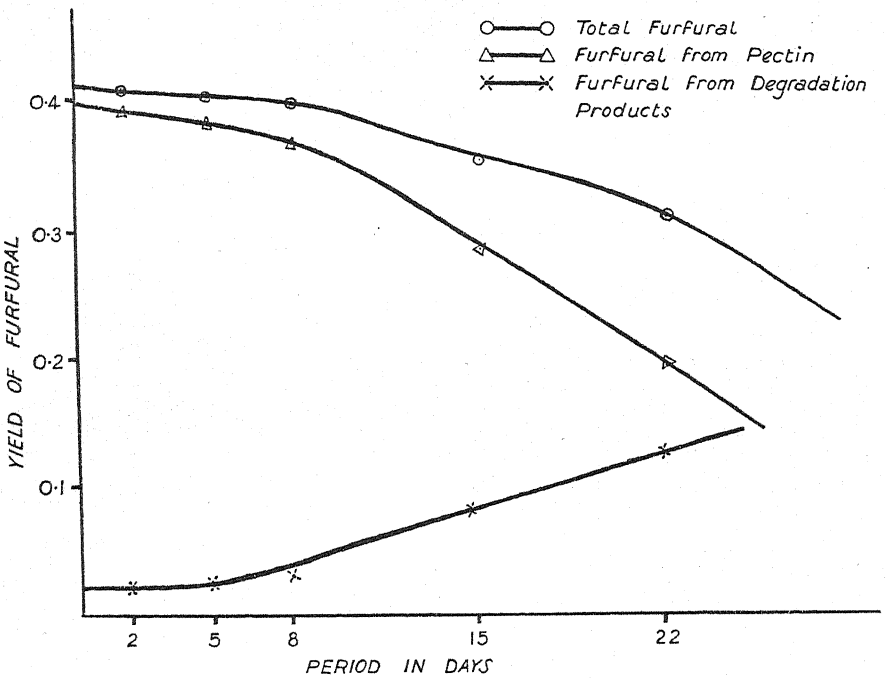


Fig. 2. Growth of *B. atrosepticus* on pectin.

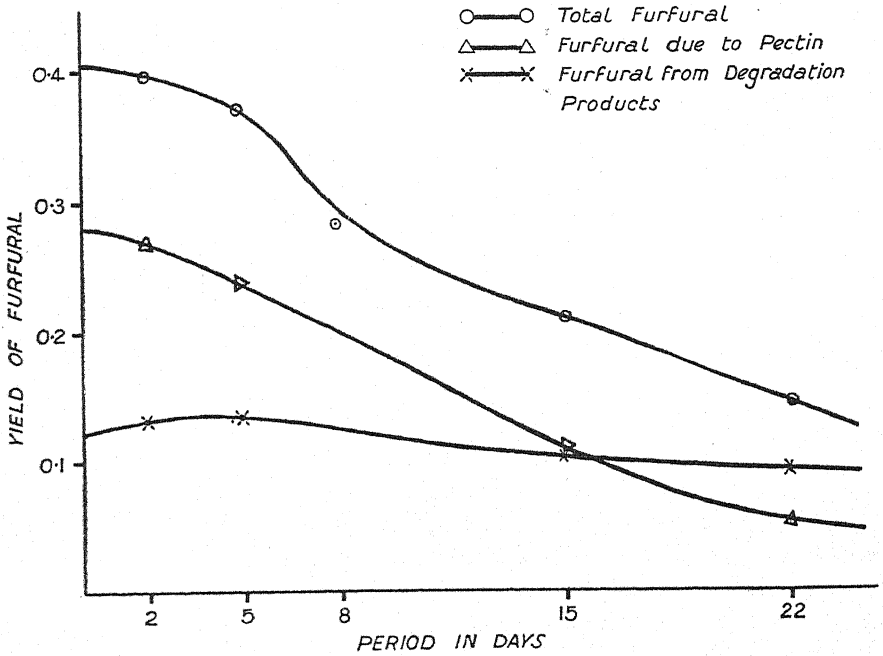


Fig. 3. Growth of *B. solanisaprus* on pectin.

III. Action of *B. solanisaprus* on pectin.

Since it was known that this organism vigorously attacks pectin, the pectin medium was autoclaved at three atmospheres for about forty minutes. This resulted in the lowering of the pectin content from approximately 2 per cent. to 1·5 per cent., and proportionately increased the amount of degradation products present.

The results are expressed on 100 c.c. of medium.

Time in Days.	Total Furfural.	Calcium Pectate Yield.	Furfural from Pectin.	Furfural from Degradation Product.	Degradation products Calculated as Pentose.
Control	0·401	1·47	0·282	0·119	0·232
2	0·392	1·39	0·266	0·126	0·245
5	0·368	1·23	0·236	0·132	0·256
8	c·284	—	—	—	—
15	0·216	0·58	0·111	0·105	0·204
22	0·146	0·28	0·054	0·092	0·179

Fig. 3 summarizes these results.

Despite the quantity of pectin degradation products present, pectin is destroyed immediately, and the products accumulate.

IV. Action of *B. subtilis* on pectin.

The medium in this case was also autoclaved so that degradation products might be present. Results expressed on 100 c.c. medium.

Time in Days.	Total Furfural.	Calcium Pectate Yield.	Furfural from Pectin.	Furfural from Degradation Products.	Degradation Products Calculated as Pentose.
Control	0·401	1·67	0·320	0·081	0·157
2	0·398	1·56	0·299	0·099	0·193
5	0·375	1·44	0·276	0·099	0·193
8	0·370	1·35	0·259	0·111	0·216
15	—	—	—	—	—
21	0·296	1·10	0·211	0·085	0·165

These results are indicated graphically in Fig. 4. They show that pectin is broken down immediately by *B. subtilis*, even in the presence of simpler degradation products. The decomposition is slow, but none the less definite, showing that the organism in question normally secretes a pectin decomposing enzyme or enzymes.

It is proposed to extend the work to a consideration of the common soil bacteria and fungi, and those of the manure heap. Slight modifications of the method will be necessary in the case of fungi, but an additional figure of significance will become available, namely, the furfural yield of the mycelium itself developed in the culture.

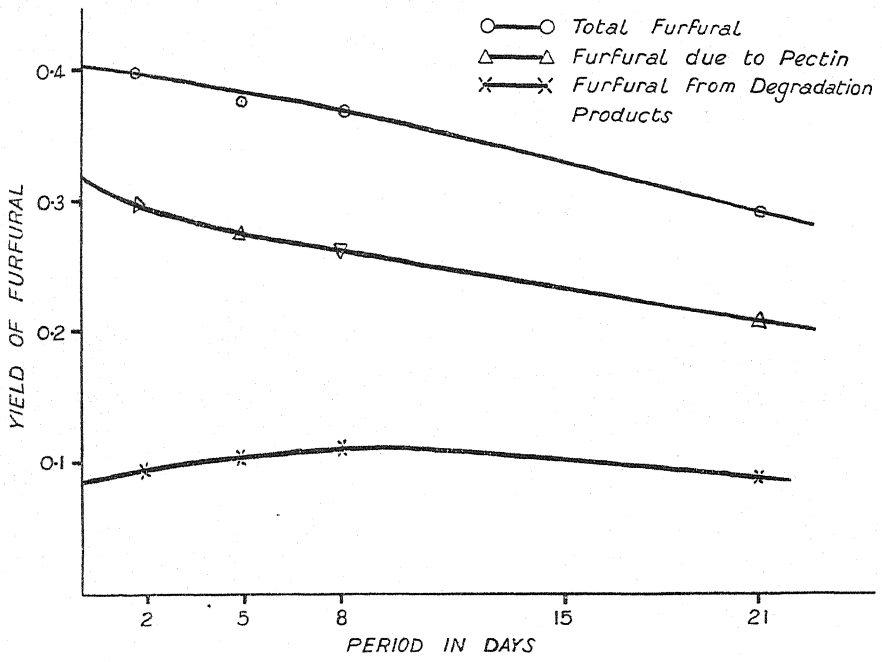


Fig. 4. Growth of *B. subtilis* on pectin.

Summary.

1. The significance of the course and nature of the micro-biological decomposition of the pectic substances in various fields is summarized.

2. A method is given by which the rate of decomposition of pectin by bacteria can conveniently be measured.

In conclusion it is a pleasure to express my indebtedness to Professor A. R. Ling for his interest and advice in the course of this work.

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Studies in the Genus Fusarium.

VII. On the Different Degrees of Parasitic Activity shown by various Strains of Fusarium fructigenum.

BY

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INTRODUCTION.

THE experiments to be described in this paper were carried out with *Fusarium fructigenum*, forms of which occur parasitically on apple fruit. During the course of artificial culture numerous saltant strains arose, making with the six original isolations upwards of forty strains in all. In a recent paper Brown (2) has shown that all these strains can be grouped into four types, on the basis of their morphological features on a standard synthetic medium. The four types are as follows:—

1. *Mycelial type*, showing strong development of aerial mycelium, and comparatively reduced sporulating capacity.

2. *Sporodochial type*, in which the aerial mycelium to a large extent disappears and is replaced by a greater or less number of pustules of spores (or sporodochia).

3. *Pionnotal type*, in which there is very little aerial mycelium at any stage, and in which the surface (and to some extent, the interior) of the culture consists chiefly of a more or less continuous layer or slime of spores.

4. *Long-spore type*, which shows resemblances to the last mentioned, but differs in that it forms a thinner and more widely spread layer or 'pionnotes' of spores, while the latter are characterized by an abnormally high degree of septation.

Certain strains show characters intermediate between those of the various types, and it is suggested by Brown (l.c.) that by long continued cultivation and separation of saltant strains as they arose, a more or less continuous series of forms could be obtained, running in small gradations from one end of the range to the other.

Of the six original isolations, five were of the mycelial and one of the sporodochial type. This experience was in agreement with that of other

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workers on *Fusarium* species, e.g. Appel and Wollenweber (1), who state that the primary isolations from diseased tissues are characterized in general by a reduced tendency to spore formation. It was suggested, therefore, that the mycelial type of strain was the most active one from the point of view of parasitic activity and that it might be possible to demonstrate, side by side with the variations in morphological features, a definite gradation in virulence between the different strain types. An investigation which was in progress in this laboratory, and a report of which has recently appeared (Horne and Gregory (7)), did in fact indicate that definite differences in parasitic power existed between certain strains. A comparison of the invasive power of nine strains of *F. fructigenum* was carried out by these workers and it appeared that as regards virulence the strains tested fell into four significantly different groups, as follows:—

1. The most active type, to which belonged five of the strains, viz. the four mycelial strains tested and one sporodochial strain.
2. A less active group, to which belonged two sporodochial strains.
3. A still less active type, represented by one pionnotal strain.
4. The least active type, represented by one long-spore strain.

Horne and Gregory's work was carried out with two varieties of apples, Cox's Orange Pippin and Bramley's Seedling, and the inoculated fruit was maintained either at 12° C. or at 3° C. These conditions of cool or cold storage naturally slowed down the rate of fungal attack very considerably, so that a long interval elapsed before the results of each experiment were forthcoming.

The present investigation was undertaken in order to examine more fully the differential behaviour of strains, as indicated in the paper just cited. It was necessary in the first instance to determine whether the variation in virulence as tested on Cox's Orange Pippin and Bramley's Seedling apples at 3° and 12° applied to other varieties of apples and to other temperature conditions. This involved the routine testing of such varieties of apples as were available throughout the year. The number of fungal strains used was also increased so as to be more representative of the various types. As an appendix to the problem here outlined, attempts of a preliminary nature were made to see whether the differences in virulence as shown between certain strains could be explained in terms of various physical or chemical features of the individual strains.

Material and Methods.

Fourteen strains of *F. fructigenum* were used in the various tests. These are set out in Table I, under their appropriate grouping. The lettering and indexing are as described by Brown in the paper already cited.

TABLE I.

Showing Grouping of Strains Used.

Mycelial Type.	Sporodochial Type.	Pionnotal Type.	Long Spore Type.
B_1	A	$B_{2\ 1}$	$B_{1,\ 1,\ 1}$
C_1	A_1	D_3	$C_{1,\ 1}$
$C_{2,\ 1}$	C_3		$C_{1,\ 2}$
D	D_2		D_1

The stock cultures of the strains were maintained on a glucose-asparagin-mineral salts synthetic medium on which their cultural appearances were well known. Any change that might arise in the course of subculturing could thus be checked. The cultures actually used for the supply of material for inoculation purposes were subcultures on potato extract agar. Tubes three to six weeks old were generally used for this purpose. As all the strains under consideration sporulate more or less freely, it was possible to carry out the inoculations with spores only. The general procedure was to touch or rub a spore mass in the culture tube with a sterile wire and then transfer to the apple. In a limited number of cases the spores were suspended in water and a measured quantity of the suspension used for each inoculation.

The apple material was in general obtained from the shops. The following varieties were tested in greater or less detail: Cox's Orange Pippin, Ribston Pippin, Bramley's Seedling, Worcester Pearmain, Newtown, Winesap, Russet, and Yellow Banana.

The method of inoculation used was that described by Granger and Horne (5) in which a shallow plug of tissue is removed from the apple by means of a cork-borer with the usual aseptic precautions, the cavity inoculated, and the plug then replaced. The margin of the plug is sealed with melted paraffin in order to protect the plug from loss of water and from extraneous contamination.

Two such inoculations, at diametrically opposite points, were made on each apple. The strains were compared two at a time and inocula of each member of a pair were placed on opposite sides of the same apple. It was known—and the results of the present investigation are confirmatory—that very great differences in resistance are shown by different apples of the same sample, and it was therefore thought likely that the factor of variable resistance could be best controlled by comparing the rates of attack on opposite sides of the same apple. That the degree of resistance of each apple is specific and that there is great variation from one apple to another has been demonstrated statistically by Gregory and Horne (6) in their recent paper.

For each comparison between two strains, eight to ten apples as far as

possible free from blemish, were generally employed. After inoculation in the manner described above, the apples were placed in a large incubator maintained at 20° C. They were examined at intervals of four or five days, and when the rot in the most advanced case showed a superficial spread of about 3 cm. the apples were removed from the incubator and the amount of rotted tissue determined for each inoculation. The duration of each experiment thus varied with the different strains and with the different apple varieties. In some cases it was about ten days, in others about a month.

The amount of rot produced can be determined with considerable accuracy by weighing the apple, scraping out the rotted portion, and weighing again. The relative activities of the strains can then be estimated in terms of the ascertained weights of rotted tissue or of data calculated from these (see later).

These inoculation experiments were carried out over two seasons, and in each from September to the following June. During the earlier part of each apple season, no trouble from extraneous organisms was met with, but towards the end of the period when the apples obtainable were very ripe there was some considerable contamination by *Penicillium* species. From time to time a whole experiment was spoilt in this way. In general, however, the *Penicillium* rot began at the stalk end of the apple, in which case it was necessary to interrupt the experiment at a somewhat early stage, so that the amounts of rot due to the *Fusarium* strains could be determined. The type of rot produced by the latter is easily distinguishable in appearance from the paler softer rot due to *Penicillium*, so that in the few cases where contamination of the plugs by the latter took place, its presence was obvious. Any apples in which such contamination by *Penicillium* had taken place or in which there was any uncertainty in delimiting the amount of rot produced by the *Fusarium* strains were discarded.

The kind of result obtained is best illustrated by quoting from a particular experiment (Table II). Three comparisons were made as follows:—

D (*m*) against D_1 (*l.s.*),
$C_{2,1}$ (*m*) against $C_{1,2}$ (*p*),
$B_{2,1}$ (*p*) against $B_{1,1,1}$ (*l.s.*).[1]

For each comparison ten apples (Newtown Pippin) were used. Examination of the table shows that some apples of each set were discarded, and this was due to contamination by *Penicillium*. The duration of the experiment was four weeks. The data represent the weights in grams of tissue rotted by the strains concerned.

[1] *m.* = mycelial type; *s.* = sporodochial type; *p.* = pionnotal type; *l.s.* = long-spore type.

TABLE II.

Apple.	Strain D.	Strain D_1.	Apple.	Strain $C_{2,1}$.	Strain $C_{1,2}$.	Apple.	Strain $B_{2,1}$.	Strain $B_{1,1,1}$.
1	20·2	0·0	1	2·8	2·7	1	0·0	0·0
2	19·4	1·2	2	16·3	14·4	2	5·9	0·9
3	3·3	0·0	3	33·3	19·3	3	0·6	0·3
4	13·6	0·6	4	30·2	10·5	4	2·7	1·4
5	12·8	0·8	5	24·4	17·3	5	0·0	0·0
6	7·4	2·3	6	15·4	4·5	6	0·8	0·0
7	1·9	0·0	7	3·0	2·1	7	4·0	0·0
8	4·7	1·4	8	0·4	1·1			
9	10·3	2·6	9	5·2	1·4			

The magnitude of the variability in resistance from one apple to another of the same sample is clearly shown in the above Table. Thus the amount of rot produced by the strain D varies from 1·9 grm. to 20·2 grm.; that of strain $C_{2,1}$ from 0·4 to 33·3 and so on.[1] Nevertheless it is seen that the strain D has produced on each apple a distinctly greater amount of rotting than has the weaker strain D on the same apple. The difference in activity between strains $C_{2,1}$ and $C_{1,2}$ is less marked. In eight cases the amount of rot produced by $C_{2,1}$ exceeds that due to $C_{1,2}$ and in one case the reverse holds good. Both the strains $B_{2,1}$ and $B_{1,1,1}$ are weakly parasitic, but apart from the two apples in which no attack took place by either strain, it is seen that strain $B_{2,1}$ causes more rot in each case than $B_{1,1,1}$.

The probability that the amount of rot produced by one strain of a pair is greater than that due to the other may be evaluated by Student's Method as described by Fisher (4). The calculated probability (P) is expressed as a decimal fraction—thus P = 0·01 means that the probability of the particular result is 1 : 0·01, i.e. 100 : 1, or in other words, that an adverse result would be obtained about once in a hundred times; similarly P = 0·3 means a probability of 1 : 0·3 or 10 : 3, so that an adverse result would be expected to appear about three times out of ten. The former figure thus indicates a high and the latter a low probability. Conventionally a probability of 20 : 1 (P = 0·05) is taken as the limit of significance. Values of P equal to or less than 0·05 indicate significant results, and the smaller the value, the greater the degree of significance. Values of P greater than 0·05 indicate differences which are not in themselves significant.

Calculated in this way, the results for Table II are as follows:

D	is more active than		D_1	P < 0·01
$C_{2,1}$	,,	,,	$C_{1,2}$	P between 0·05 and 0·02
$B_{2,1}$	,,	,,	$B_{1,1,1}$	P ,, 0·1 and 0·05.

[1] It may be remarked that none of these *Fusarium* strains—not even the most active of them—is comparable in rotting activity on apple fruit with such a virulent parasite as *Monilia fructigena*. While there is a great range of activity within the *Fusarium* strains, none of them is an extremely active parasite. It is under such conditions, therefore, i.e. with parasites of only moderate activity, that one would expect that variation in susceptibility of the host would have the greatest effect on the amount of attack produced.

Thus the first two comparisons indicate significantly different degrees of parasitic activity; in the case of the third comparison, a difference of activity is indicated, but this particular result in itself is not statistically significant.

In calculating the probability that the parasitic activity of one strain is greater than that of another, one may use directly the data observed experimentally, that is, the amounts of rotted tissue produced. Gregory and Horne (6), however, have shown that when the data of weight are converted into terms of rate of radial advance, a distribution is obtained which confirms more closely to that of a normal distribution curve, and they point out that it is only to such normally distributed variates that the usual statistical methods are strictly applicable. In the present paper, the probability values have been calculated on the basis both of the weights of rotted tissue and of the rates of radial advance, the latter being determined from the formula given by Gregory and Horne (l.c. p. 431). The degree of significance of the various differences varies to some extent according as the calculations are based on weights of rotted tissue or rates of radial advance, but as will be shown more fully later, the main results are the same whichever method of calculation is adopted. The general effect of using Gregory and Horne's method is to increase the percentage of significant results. Thus, in a series of 44 experiments, it was found that 21 gave significant and 23 gave non-significant differences when the basis of calculation was the weight of rotted tissue. On the other hand, when the calculation was carried out by Gregory and Horne's method the corresponding figures were 27 and 17. More fully, the analysis of the results was as follows:

21 experiments gave significant differences according to either method of calculation.

17 experiments gave non-significant differences according to either method of calculation.

6 experiments which were non-significant according to the first method become significant when calculated by the second method (G. and H.).

In no case did a result which was significant by the first method become non-significant when calculated by Gregory and Horne's method.

The above results are in agreement with Gregory and Horne's observation that a more normal distribution of the variates is obtained when the latter are expressed in terms of radial advance and not of amounts of tissue rotted.

Comparison of Activity of Different Types of Strain.

Comparative tests of virulence have been carried out with pairs of strains as follows:—

Mycelial	type versus	long-spore	type.
„	„ „	pionnotal	„
„	„ „	sporodochial	„
Sporodochial	„ „	pionnotal	„
„	„ „	sporodochial	„
Pionnotal	„ „	long-spore	„

The results are set out in Tables III–VIII.

Mycelial type v. *Long-spore type.*

Considerable attention was paid to a comparison of these two types, which are respectively the most active and least active of the series. The object of the investigation was in the first instance to test whether certain strains which arose by saltation were definitely less virulent than their parents. The feeble activity of the long-spore type of strain had been indicated in preliminary experiments, and therefore an extended comparison was made of two mycelial strains with saltants of the long-spore type derived from them.

The results of seventeen such tests are given in Table III. The second column gives the number of apples employed in each test. Under the heading 'Activity' is given the result of the comparison, e. g. by '$D > D_1$' is meant that the average amount of attack produced by D is greater than that produced by D_1, and so on. In this column the mycelial type of strain is placed first in each case. The fourth and fifth columns give the probabilities that the results stated in the third column possess real meaning, the notation adopted being that explained above (p. 249). The first series of probabilities (P_1) are calculated directly from the weights of tissue parasitized; the second series (P_2) from the corresponding rates of radial advance of the fungus into the apple tissue.

TABLE III.

Variety.	No. of Apples.	Activity.	P_1.	P_2.
Yellow Banana . .	4	$D > D_1$	0·2 −0·1	0·05 −0·02
Winesap . . .	7	„	0·1 −0·05	< 0·01
Cox's Orange Pippin .	7	„	0·05−0·02	< 0·01
Bramley's Seedling .	9	„	0·05−0·02	< 0·01
Newtown . . .	9	„	0·01	< 0·01
„ . . .	9	„	< 0·01	< 0·01
Russet	6	„	0·05−0·02	0·05
Worcester Pearmain .	7	„	0·05	< 0·1
Cox's Orange Pippin .	6	$C_1 > C_{1,1}$	< 0·01	< 0·1
Bramley's Seedling .	6	„	0·1 −0·5	0·05
Worcester Pearmain .	8	„	0·05−0·02	< 0·01
Newtown . . .	10	„	< 0·01	< 0·01
„ . . .	10	„	< 0·01	< 0·01
Winesap . . .	8	$C_1 > B_{1,1,1}$	< 0·01	< 0·01
Cox's Orange Pippin .	7	„	< 0·01	< 0·01
Bramley's Seedling .	10	„	< 0·01	< 0·01
Russet	7	„	0·4	0·3

It will be seen that in all the seventeen tests the mycelial strain is more active than the long-spore strain. According to the first method of calculation (P_1) thirteen of the tests are statistically significant, the usual standard of significance being taken as a probability of 20 : 1 or over. The number of significant results in the P_2 column is sixteen.

If one assumes that the data for the different strains and for the different apple varieties can be combined into a single result, one can determine the probability that the mycelial type of strain is more active than the long-spore type when tested on apple fruit generally. Whether one uses for this purpose the figures given in the P_1 column or the P_2 column, or whether one takes the lower value in each case, a figure is obtained which indicates an enormously high probability that the mycelial type of strain is more virulent than the long-spore type.

It should be noted that strains D_1 and $C_{1,1}$ arose directly by saltation from strains D and C_1 respectively. Table III shows distinctly that in each case the saltant strain is much less active parasitically than the parent.

Mycelial type v. *Pionnotal type.*

The results are set out in Table IV in the same manner as in the preceding Table. In the third column the mycelial strain is placed first in each case.

TABLE IV.

Variety.	No. of Apples.	Activity.	P_1.	P_2.
Bramley's Seedling .	9	$C_{2,1} > B_{1,1}$	0·05 – 0·02	0·01
Cox's Orange Pippin .	7	,,	< 0·01	< 0·01
Bramley's Seedling .	7	$C_{2,1} > C_{1,2}$	0·1	0·3
Cox's Orange Pippin .	5	,,	0·4	0·3
Worcester Pearmain .	9	,,	0·1 – 0·05	0·1 – 0·05
Newtown . . .	10	,,	0·02 – 0·01	< 0·01
,, . . .	9	,,	0·05 – 0·02	0·05 – 0·02

Again it is seen that in all cases the mycelial strain is the more active, but the proportion of non-significant results is greater. If, as before, the results are combined, one finds a very high probability (much greater than 100 : 1) that the mycelial type of strain is more active than the pionnotal type.

Mycelial type v. *Sporodochial type.*

The results are given in Table V, the mycelial strain being placed first in column 3.

TABLE V.

Variety.	No. of Apples.	Activity.	P_1.	P_2.
Bramley's Seedling .	8	$B_1 > C_3$	0·3	0·4
Cox's Orange Pippin .	7	$B_1 = C_3$	—	—
Bramley's Seedling .	10	D > A	0·02 – 0·01	< 0·01
Cox's Orange Pippin .	7	,,	0·05 – 0·02	0·05 – 0·02

In this case, three of the results gave the mycelial strain as the more active; in the fourth, the mycelial and sporodochial strains showed equal amounts of attack. The greater activity of strain D, as compared with A, is clearly indicated. On the other hand the mycelial strain B_1 does not appear to be appreciably more active than the sporodochial strain in C_3. It is interesting to note that Horne and Gregory, who tested the four strains of Table V (loc. cit., p. 458), placed them in order of diminishing virulence as follows—D, B_1, C_3, and A. Though the data of Table V are not extensive, it is clear that the indications are in complete agreement with Horne and Gregory's results. When the individual probabilities as given in Table V are combined, the probability that the mycelial type is more active than the sporodochial is much greater than 100 : 1, viz. in the neighbourhood of 400 : 1, even when one selects as between columns P_1 and P_2 the individual probability figures which are least favourable to the result indicated.

Sporodochial type v. *Pionnotal type.*

The results of the six comparisons carried out are given in Table VI, two sporodochial strains A and D_2 being tested against a pionnotal strain D_3.

TABLE VI.

Variety.	No. of Apples.	Activity.	P_1.	P_2.
Russet	5	$A > D_3$	0·5	0·5
Bramley's Seedling .	8	$D_2 > D_3$	0·05 – 0·02	0·05 – 0·02
Cox's Orange Pippin .	6	,,	0·1	0·1 – 0·05
Worcester Pearmain .	8	,,	0·2 – 0·1	0·5
Newtown	10	,,	0·3	0·3 – 0·2
,, ...	8	$D_2 < D_3$	0·5	0·4

Here it is seen that the sporodochial strain is the more active in five cases out of six, the remaining case offering an exception. Furthermore, a significant difference is only shown in one of the tests. It is indicated, therefore, that the two types of strain are nearer to each other in parasitic activity than those which have been dealt with in Tables III–V. When the six results are combined, the probability that the sporodochial type is more active than the pionnotal is:

(1) just greater than 100 : 1 when the values given in the P_1 or in the P_2 column are used;

(2) between 20 : 1 and 50 : 1, but nearer the latter, when one uses for summation the less favourable of each pair of figures given in columns P_1 and P_2.

The data contained in Table VI are therefore sufficient to prove the greater virulence of the sporodochial strains tested as compared with the pionnotal strains, but it is clear that the evidence is not so overwhelming as in the tests recorded in Tables III–V.

Sporodochial type v. *Sporodochial type.*

A number of comparisons were made in which a sporodochial strain A was tested against strain A_1, which is a saltant from the former, and which is also of sporodochial type. The two strains differ to a slight extent in sporulating capacity and in colour production on suitable media. The data are given in Table VII.

TABLE VII.

Variety.	No. of Apples.	Activity.	P_1.	P_2.
Bramley's Seedling .	7	$A < A_1$	0·4	0·5
Cox's Orange Pippin .	7	$A > A_1$	0·6	0·6
Worcester Pearmain .	7	$A < A_1$	0·6	0·6
Newtown . . .	10	$A < A_1$	0·6	0·6
„ . . .	9	$A > A_1$	0·1	0·05 – 0·02

In this case it will be observed that out of five tests, A is less active than A_1 in three cases, and more active in the remaining two. Again, there is only one result which in itself is significant, and then only when the calculation is based on the rates of radial advance.

When the results of Table VII are combined, it is found that strain A is more active than A_1, but that the probability of this result being real is only about 3 : 1, even when the figures *most favourable* to the greater activity of A are chosen from columns P_1 and P_2. Thus the data are consistent with the view that strains A and A_1 are not appreciably different in parasitic vigour.

Pionnotal type v. *Long-spore type.*

The available data are contained in Table VIII.

TABLE VIII.

Variety.	No. of Apples.	Activity.	P_1.	P_2.
Bramley's Seedling .	8	$B_{2,1} > B_{1,1,1}$	0·2 – 0·1	0·05
Cox's Orange Pippin .	7	„	0·2	0·4
Worcester Pearmain .	10	„	0·2	0·3 – 0·2
Newtown . . .	7	„	0·1 – 0·05	0·05
„ . . .	10	„	0·01	< 0·01

The pionnotal strain $B_{2,1}$ proved to be more active than the long-spore strain $B_{1,1,1}$ in all five tests. In only one case, however, was the difference in virulence clearly significant.

When the five results shown in Table VIII are combined, the probability that the pionnotal strain $B_{2,1}$ is more active than the long-spore strain $B_{1,1,1}$ is found to be much greater than 100:1, and this is true whether the individual probabilities are taken from the column P_1 or from P_2, or whether the least favourable selection is made from the two columns.

The general nature of the results contained in Tables III–VIII may be summarized as follows: the mycelial type of strain is the most actively parasitic, then follow in order the sporodochial, pionnotal, and long-spore types. The difference in virulence between strains of *Fusarium fructigenum* is most readily demonstrable when members of the mycelial group are compared with those of the long-spore group, as is clear from the large percentage of individually significant results shown in Table III. None the less it has been possible to demonstrate significant differences in virulence between consecutive members of the series of strain types. It is noteworthy that in the series of tests in which two strains of the same type were compared, no evidence of a difference in parasitic power was obtained. There is, however, no reason for thinking that all the strains of a particular type are of equal virulence, but it is obvious that the demonstration of such differences might require extensive experimentation with material specially selected from the point of view of uniformity.

Certain varieties of apple fruit were found to be more rapidly attacked than others, e. g. Cox's Orange Pippin than Bramley's Seedling, and Newtown Pippin more than Ribston Pippin. Whether these differences were due to inherent properties of these varieties, or merely to different degrees of maturity, or different cultural conditions, could not of course be determined with the material available. It was clear, however, that there was no indication of selectivity on the part of the fungal strains used; that is, the strains which were among the most active on one particular variety of apple were also among the most active on other varieties, and similarly for the less active strains.

Elimination of certain Factors as Influencing Amount of Attack.

A number of experiments of a preliminary nature were carried out with the object of attempting to analyse the factors responsible for the different degrees of virulence shown by these *Fusarium* strains. From this point of view the results were of an entirely negative character, but they are interesting, inasmuch as they indicate that the differences of virulence are intrinsic to the various strains, and are not due to any conditions arising from the experimental methods employed.

The first point investigated was whether inocula consisting of mycelium

were more active in producing attack than sporal inocula. If this were so, the greater activity of the mycelial types might be due to the accidental inclusion of pieces of mycelium with the spores which were used as inoculum. For this purpose eight Newtown apples were inoculated on one side with spores only, and on the other side with mycelium only of the strain A (*sp.* type), and a second series of apples inoculated in the same way with strain C_1 (*m.* type). With both strains the average amount of attack produced by spores was greater than that produced by mycelium. In neither case was the difference of significant amount, nor was a significant difference indicated when the results were combined. If there is any real difference between the two kinds of inocula, it is in favour of the spores, and therefore the greater virulence of the mycelial type of strain is not to be explained on the basis of accidental inclusion of pieces of mycelium in the inocula used.

The possibility that the number of spores introduced as inoculum might influence the amount of attack was also investigated. In the case of the long-spore type of strain, which is the weakest, the spores occur in a thin layer on the surface, but a great number are formed within the medium, and are therefore not available when the culture is rubbed by the inoculating wire. There was therefore a possibility that the weakness of this type of strain might be due to inoculation in general with a small dose of spores.

An experiment to test the effect of spore concentration on rate of attack was carried out with the mycelial strain D, and the long-spore saltant D_1. Suspensions of the spores of each strain were made in sterile water, such that the two suspensions appeared equally turbid. This concentration was designated N, and by means of a sterile pipette a series of dilutions N/2, N/4, N/10, and N/100 were made. Loopfuls of the most dilute of these suspensions were examined under the microscope, and were found to contain approximately the same number of spores in each case. The apples were inoculated as follows:

Set I. Macroscopically visible mass of spores on one side; loopful of suspension N on the other.

„ II. Loopful of N on one side, of N/2 on the other.
„ III. „ N/2 „ N/4 „
„ IV. „ N/4 „ N/10 „
„ V. „ N/10 „ N/100 „

The average amounts of attack produced in twenty-five days are given in Table IX.

TABLE IX.

Strain.	Mass of Spores.	N.	N/2.	N/4.	N/10.	N/100.
D	14·1 grm.	13·9	10·6	—	18·6	9·1
D_1	2·6	2·2	3·3	4·1	5·5	3·5

It is certain that one loopful of the concentration N/100 represents a smaller dose of spores than was employed in the tests described earlier in this paper, and similarly that the large mass of spores at the other end of the series of concentrations is an abnormally high dosage. Table IX shows that over a very wide range of concentrations of spores in the inoculum the amount of attack produced by strain D_1 is regularly much less than that produced by D under the same conditions. The weakness of strain D_1, therefore, cannot be ascribed to any tendency on the part of the experimenter to use too few spores in the inocula.

These experiments, therefore, indicated that differences in virulence observed were independent of any circumstances incidental to the experimental routine. Further tests were now made to see whether the virulence of a weak type of strain could be increased by certain kinds of treatment of the spores previous to inoculation.

The microscopic appearance of the spore varies according to the type of medium on which the fungus has been grown, as has been shown in detail by Brown and Horne (3). In particular, the spores produced on media with a high carbon-nitrogen ratio are of a very granular nature, that is, are full of reserve food material. Thus it might be considered that they are well equipped for germinating strongly, and therefore for initiating attack. An experiment was carried out to test this point.

Cultures of strains D (*m.*), A (*sp.*), $B_{2,1}$ (*p.*), and D_1 (*l.s.*) were grown on the following three media:

I. Potato agar.

II. Synthetic agar in which the carbohydrate : nitrogen ratio was 1 : 1.

III. Synthetic agar in which the carbohydrate : nitrogen ratio was 60 : 1.

Table X shows the results obtained in terms of average amount of attack per apple of eight Newtown Pippins.

TABLE X.

Strain.	Medium I.	Medium II.	Medium III.
D	3·7	5·0	7·1
A	3·6	4·3	3·5
$B_{2,1}$	2·7	2·1	0·9
D_1	1·9	1·3	1·9

The weak strains $B_{2,1}$ and D_1 are seen to be weak on all the media tried; and conversely for the more active strains D and A. The only difference which reaches a 20 : 1 probability is that between $B_{2,1}$ on Medium I as against Medium III. When the results are combined, they are as follows:

Spores from Medium I are less active than those of Medium II. $P = 0{\cdot}9$.

" " I " " " III. $P = 0{\cdot}4$.

" " II " " " III. $P = 0{\cdot}8$

The differences are thus negligible, and afford no suggestion that the virulence of the spores used for inoculation is associated with the nature of the medium from which they are taken.

In many cases where a weak strain is used for inoculation, it is found that no demonstrable amount of attack has taken place. Repeated observation has shown that failure of the spores to produce attack is not due to failure to germinate. Nevertheless there is a possibility that such spores may be somewhat retarded in their germination, so that the wounded surface of the apple fruit is able, in the interval, to protect itself in some manner, e. g. by more or less drying, or by a certain amount of cork formation. If such were the case one would expect that the amount of attack produced by a weak strain would be appreciably increased if spores, which had already germinated, were used in the inocula. An experiment carried out in the usual way to test this point actually gave a somewhat smaller amount of rot produced by the germinated spores. In a somewhat similar experiment, the activity of spores of a weak strain was tested under two contrasting conditions: (1) where the spores were suspended in water; (2) where the spores were suspended in a nutrient. Here also no appreciable difference was observed in the two cases. These experiments indicate that even when provision is made for the vigorous germination of the spores of a weak strain, the latter are still unable to bring about a strong attack of the plant tissue.

Summary.

1. From a quantitative study of the rate of attack of apple tissue by a number of strains of *Fusarium fructigenum*, it is shown that the latter exhibit at least four grades of parasitic vigour.

2. The degree of virulence shown is correlated in a general way with the morphological character of the strain. Thus it is greatest in the type of strain which shows the greatest tendency to the mycelial type of growth. The types of strain which are described as 'sporodochial', 'pionnotal', and 'long-spore' follow in the order named.

3. No evidence of selective activity has been obtained. The order of the strains, as regards virulence, is much the same on one variety of apple as on another.

4. The activity of each strain appears to be independent of such factors as the quantity of inoculum used, the kind of inoculum (sporal or mycelial), and the physiological state of the inoculum (presence of reserve food material in the spores, &c.).

In conclusion, I wish to thank Professor W. Brown for suggesting the problem to me in the first instance, and for his continuous interest and help throughout the course of the work.

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The Transmission of Inhibition through Dead Stretches of Stem.

BY

R. SNOW.

With two Figures in the Text.

FROM certain experiments already reported (3) it was concluded, in agreement with most previous investigators, that a growing shoot apex inhibits its axillary buds not by withdrawing any nutritive substances from them, but by transmitting some influence to them. It was found that this influence would pass across a protoplasmic discontinuity between two cut surfaces of tissue that were pressed tightly together (3, Expt. (8)). This experiment made it appear that in the transmission of inhibition the movement of a soluble inhibiting substance is involved. But the experiment seemed not quite conclusive, since the protoplasms at the two cut surfaces might have fused together in some places, though examination did, indeed, make it very unlikely that they had done so. It seemed that further light would be thrown on the question, if an experiment could be arranged to test whether inhibition can be conducted with the transpiration stream through the vessels of a dead zone of stem.

It has been shown by Newton Harvey (2) that if a zone of the epicotyl of *Phaseolus* is killed by steaming, inhibition does not pass down through it from the apex, for axillaries grow out below the dead zone, while the apex continues to grow above it. His experiment serves well to test whether inhibition is brought about by a draining away of nutriment towards the apex, or whether by some process conducted away from the apex; and it provides valuable evidence in favour of the latter theory. But it does not determine whether the conduction depends on the movement of a soluble substance or not. For such a substance, even were it able to enter the vessels, clearly could not pass downwards in the transpiration stream, since this normally moves upwards only. Such a substance might none the less be able to pass upwards through a dead zone, and accordingly the following experiment was devised to test whether it can do so.

Young seedlings of *Vicia Faba*, of the race 'Johnson's Long Pod', were decapitated in the epicotyl, so that the two axillaries of the cotyledons

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grew out. Those seedlings were then selected in which the axillaries were nearly equally strong, and the shorter of the two was decapitated above its second leaf (cf. Fig. 2). A bud was allowed to remain in the axil of one of the two leaves of this shoot, and the bud in the axil of the other leaf was removed. The remaining bud on the decapitated shoot still did not grow out even after three or four weeks, or not more than 1 mm. at most. Clearly it was inhibited by the apex of the longer shoot, for if that shoot also was decapitated, then this bud was found to be growing out strongly after about eight days, or less.

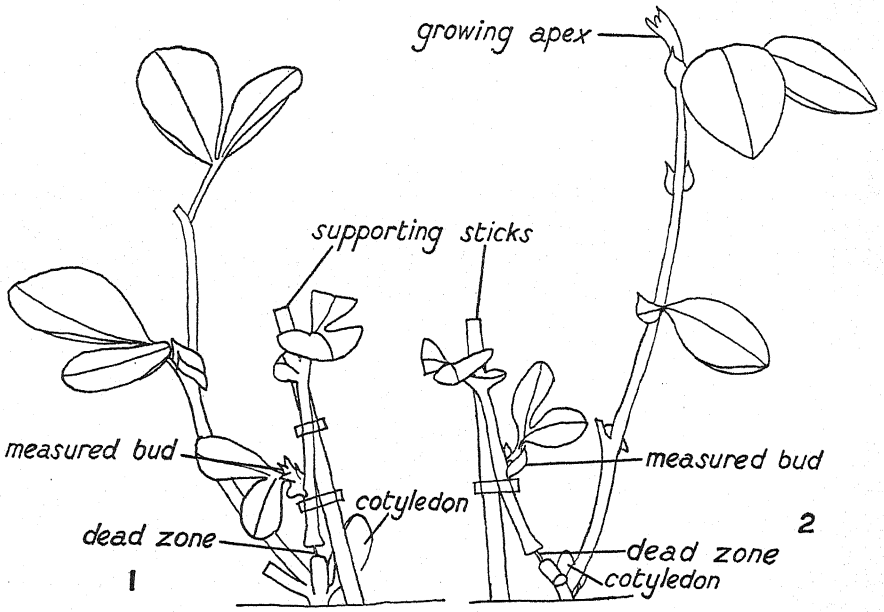
The above arrangement, therefore, made it possible to experiment on an inhibition that was travelling upwards instead of downwards. The next step was to determine whether inhibition would still pass upwards through a dead zone. For this purpose a zone of about 4 mm. near the base of the decapitated shorter shoot was killed with a hot glass rod, care being taken not to char the tissues or boil the water in them. This method was found much more convenient than killing a zone with a jet of steam. The shoot had previously been firmly tied to a stick above, and the dead zone was painted over with vaseline. This arrangement constituted the experiment, while controls were provided by cutting off the apex of the longer shoot also and removing all axillary buds from the whole plant, except the one to be measured (see Figs. 1 and 2, which represent the plants seventeen days later). The point was now to compare the growth of the bud above the dead zone in the experiments with its growth in the controls.

The leaves above the dead zones usually remained turgid for three weeks or more, after which time they wilted, probably because the dead vessels became blocked. Just above the zones swellings appeared, after an average time of about fourteen days, and from these afterwards roots developed, as in Newton Harvey's experiment.

The plants were grown in a mixture of sand and elm sawdust, and were kept in a greenhouse in which a fairly high rate of evaporation was maintained, in order to hasten the movement of the transpiration stream through the dead zone towards the measured buds. With the same object, buds in the axils of fairly well-developed leaves were selected for measurement. But the greatest rate of evaporation that could safely be allowed, without risk of causing the leaves above the dead zones to wilt, was roughly that at which a soaked piece of red blotting-paper, laid beside the plants, dried in three hours. The right conditions were most easily obtained in early spring and autumn, for in hot summer weather the leaves above the zones wilted too soon.

The plants were operated on in pairs on the same day, or occasionally on successive days, and great care was taken to have the experiment and control of each pair approximately equal in the area of leaf above the dead zone, in the vigour of the whole plant, and in the initial size of the buds to

be measured. These buds were usually only from 1·0 mm. to 1·5 mm. long. Larger buds tended to grow out too readily, and were avoided, since it was thought that inhibition, even if it could pass the dead zone at all, might very probably be greatly weakened in doing so.



Figs. 1 and 2. Fig. 1, a control plant, and Fig. 2, an experimental plant, both seventeen days after operation.

The scorched zones were always examined by sections at the end of tne experiment, and were found to be dead all through. In fact they shrank within the first day to little more than thin strands of dead xylem. For further security, three more plants were similarly scorched, and examined by sections after one day. These scorched zones also were found to be dead all through.

The following table gives in mm. the growth made by the measured buds during the first sixteen days. Plants in which the leaves above the dead zones wilted after less than sixteen days are omitted. The letter 'u' means that the bud was in the axil of the upper leaf: in the other plants it was in the lower axil.

The mean growth in the 26 controls was 3·37 mm. ±·25, and in the 27 experiments 1·54 mm. ±·13. The difference of the means is 1·83 mm. It is 4·36 times its own standard deviation and fully significant.

Thus the bud on the decapitated shoot, above the dead zone, grew out much less rapidly if the apex of the other shoot remained, than if that apex also was removed.

TABLE.

Growth of Buds above Dead Zones in Sixteen Days in Millimetres.

Pairs of Plants.	Controls (26 Plants).	Experiments (27 Plants).	Pairs of Plants.	Controls.	Experiments
1	3·50	—	16	3·50	0·75
2	4·25	1·00	17	—	1·75
3	3·50	1·25	18	4·75	2·25
4	8·75	1·00	19	3·25	1·50
5	2·50	4·00	20	6·00	2·50
6	—	1·00	21	—	1·00
7	2·00	1·25	22	2·75 (u)	0·75 (u)
8	2·00	0·75	23	1·50 (u)	0·50 (u)
9	4·75	3·25	24	7·75	1·50
10	1·50	2·50	25	2·00 (u)	0·25 (u)
11	3·50	0·25	26	2·00	2·75
12	0·50	0·50	27	4·50 (u)	—
13	1·25	0·75	28	3·75 (u)	0·50 (u)
14	2·25	2·00	29	1·25	2·50
15	4·25	—	30	—	2·50 (u)
				mean = 3·37 ± 0·25	mean = 1·54 ± 0·13

The interpretation proposed is that an inhibiting substance, formed by the growing apex of the longer shoot, is transported through the living tissues and leaks into the vessels just below the dead zone. It is then drawn up with the transpiration stream through the dead zone and leaks out into the living part of the shorter shoot, where it inhibits the growth of the axillary bud. This leaking into the vessels can be understood; for in one similar plant, examined after two days, it was found that just above and below the completely dead zone, dead xylem was for 1 mm. or more in contact with living soft parenchyma of the pith. It does not follow that the inhibiting substance enters the vessels in a normal shoot.

But it is necessary to consider whether the results could be interpreted in any other way. It is sufficiently clear that no 'dynamic' process of inhibition, such as is contemplated by Child (3), was conducted across the shrunken dead zone; and it was concluded previously (3) that in general inhibition is not brought about by withdrawal of nutriment. But even so, it must still be considered whether with the present peculiar experimental arrangement, the weaker growth of the buds on the experimental plants may be due to their being less well nourished.

The areas of the leaves above the dead zones were kept roughly equal in experiments and controls. And it seems to have been mainly from the photo-synthetic products of these leaves, unable to escape downwards through the dead zones, and not from the cotyledons below, that the measured buds obtained their organic nutriment. For from one of the controls recorded above (number 15) the cotyledons were removed after one day, and yet the bud above the dead zone grew strongly. It grew 4·25 mm. in 16 days and 6·25 mm. in 24 days. None the less, care was taken to

select plants with cotyledons large enough to last throughout the 16 days. In only two controls (numbers 15 and 16) and two experiments (16 and 22) did they fail to do so. In two more experiments (18 and 30) and one more control (18), they lasted for just 16 days, and in all other plants they lasted for longer than 16 days. It will be considered below whether their contents were more easily available in the controls.

As to water, it may be suggested that the parts above the dead zones were better supplied in the controls, since in the experiments the growing long shoots, which were competing with them for water, developed after some time a larger leaf area. But, as against this, the roots were kept always abundantly supplied with water, and the difference in leaf area was only slight. For the long shoots of the controls were decapitated close to the tip, so that they retained their two or three expanded leaves, and at first were equal in leaf area to those of the experiments. Only after a week or more did the long shoots of the experiments expand one, or sometimes two more leaves.

This slight possible source of error was avoided in the last twelve pairs of plants by removing the developing leaves of the long shoots of the experiments at a very young stage. Yet the difference in the growth of the buds on the short shoots did not diminish. For the mean growth in the controls of the last twelve pairs alone (10 plants) was 3·47 mm. $\pm$0·43, and in the corresponding experiments (11 plants) it was 1·48 mm. $\pm$0·18. The difference of these means is 1·99, and its standard deviation is 0·69. The difference is therefore 2·88 times its own standard deviation, and may reasonably be considered significant.

The times that elapsed before the short shoots wilted were actually about equal for controls and experiments. The mean time for 28 controls was 24·0 days, and for 26 experiments 23·7 days.

Finally it may be suggested that the salts taken in by the roots from the soil or passing out from the cotyledons were less easily available to the buds on the short shoots of the experiments, because in these the growing apices of the long shoots were consuming salts at the same time. The same suggestion may be made for the organic contents of the cotyledons, though it is doubtful whether any very great amount of these reached the measured buds at all. But the only way in which salts could pass up through the shrunken dead zone into the short shoots was by movement in mass with the transpiration stream. Now from what has been said above, it is clear that the rate of flow of the transpiration stream must have been roughly equal for experiments and controls in the last twelve pairs of plants (which are significant by themselves), and very nearly equal, if not quite, in the earlier pairs. Thus the amount of salts passing up through the vessels of the dead zone must also have been about equal, unless their concentrations in the water of these vessels were different. But the concentrations would

not have been different, unless after the salts had entered the conducting channels supplying the short shoots, and before they had travelled the very short distance to the dead zones, some part of them had in the experiments diffused out again and moved across to the conducting channels supplying the growing apices of the long shoots. It seems excessively unlikely that this occurred to an extent sufficient to bring about the results recorded, since the long shoots were directly and abundantly supplied with salts from cotyledons and roots through their own intact conducting channels. Moreover, in the last twelve pairs of plants, the consumption of materials by the apices of the long shoots was reduced by removing the young leaves as they reached a length of about 15 mm., and yet the difference between experiments and controls did not diminish.

Some indication also of the actual nutritive conditions in the short shoots may be obtained from the times after which swellings first appeared above the dead zones. These times were first systematically recorded for the later pairs of plants, from number 19 onwards. Among these plants, the mean time for 10 controls was 13·0 days $\pm$0·30, and for 11 experiments 13·4 days $\pm$0·33. These mean times are practically the same, for the slight difference is not significant. It is possible, however, that in the earlier pairs of plants there may have been a small significant difference in this respect, and, further, it cannot be assumed that the nutritive materials needed for the swellings are necessarily the same as those needed for the buds.

It hardly seems possible to explain the results otherwise than by an inhibiting substance. And, furthermore, even if it were possible to explain them by nutritive differences, any attempt to extend such an explanation to the results observed in similar plants without a dead zone would lead to contradictions. For in the controls, the buds above the dead zones grew out (as was often noted) less rapidly than on similar control plants that had had no zone of stem killed. Therefore, the nutritive conditions above the dead zones were, as might be expected, less favourable than in similar shoots without a dead zone. But on turning to the experiments, we find that here also the buds above the dead zones did usually grow out, though only very slowly, whereas similarly situated buds on similar plants without a dead zone were so strongly inhibited that they practically did not grow at all. (The mean growth of the buds of ten such plants after sixteen days was 0·35 mm., and the greatest growth of any bud was 0·75 mm.). How then could this be explained by nutritive differences?

The hypothesis of an inhibiting substance easily covers all the facts, including the one mentioned last. Inhibition can pass upwards, but not downwards, through a dead zone: therefore some of the inhibiting substance finds its way into the transpiration stream. But in passing through such a zone inhibition is diminished in strength. It is therefore concluded that the way by which the inhibiting substance chiefly travels is through the

living tissues, through which indeed, it has previously been shown to travel (3).

The results of this paper tend also to render unnecessary the suggestion, made previously, that the conduction of inhibition may be a complex process into which the movement of an inhibiting substance enters as one link only. For if this substance can effectively travel for about 4 mm. through a dead zone, it becomes easier to believe that in living tissues it may effectively travel all the way from apex to axillary bud.

The chief difficulty for the theory of an inhibiting substance is one raised by Child and Bellamy (1, p. 265), namely why the growing apex is not itself inhibited by such a substance, if it produces one. But it does not seem at all impossible that an answer to this question may be found.

Summary.

1. If a decapitated shoot of a plant of *Vicia Faba* has a zone near its base killed by scorching, an axillary bud above this zone can still be inhibited by the apex of a second shoot of the same plant.

2. The interpretation is discussed, and it is concluded that an inhibiting substance, coming from the apex of the second shoot, is drawn up through the dead zone with the transpiration stream.

The Department of Botany, Oxford.
October 8, 1928.

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An Investigation of the Changes which take place in the Chemical Composition of Pears stored at Different Temperatures, with Special Reference to the Pectic Changes.[1]

BY

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With seven Figures in the Text.

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I. INTRODUCTION.

THE work of this laboratory has principally been concerned with the investigation of chemical change in apples in relation to storage life. The short storage life of pears as compared with most apples, and other obvious differences, made it seem probable that there were important differences in the chemical changes in the two fruits. A preliminary study has therefore been made of the effect of storage at different temperatures upon the changes in the constituents of Conference pears, and, in particular,

[1] Thesis accepted for Ph.D. Degree of the University of London.

the part played by the pectic compounds of the cell-wall in the economy of the fruit, has been investigated. Carré (6) has shown that in apples during ripening an insoluble pectic substance (pectose) is progressively converted into a form soluble in cold water (pectin), and that decomposition of these substances to simpler compounds only takes place when break-down of the fruit sets in. It is the generally accepted view that the conversion of 'pectose' to 'pectin' is responsible for the softening of the fruit, and the very marked softening exhibited by pears during ripening and break-down suggested that the pectic changes were very important in determining the length of the storage life.

The present paper, therefore, gives the results of (1) A preliminary set of analyses of Conference pears stored at 12° C. and 1° C., which formed the basis of the plan upon which later investigations were carried out.

(2) Analyses of Conference pears stored in two successive years at 4° C. (1925) and 5° C.[1] and 1° C. (1926).

II. Previous Work on the Storage and Chemical Composition of Pears.

A considerable literature exists on the subject of the storage of pears, and a certain amount on their chemical composition. Some investigators have been chiefly concerned with the comparison of the chemical composition of different varieties, and of different parts of the same fruit. Of these investigations it is sufficient to note here that Dunbar and Bigelow (10) found that citric acid was the predominating acid in Bartlett, Idaho, Le Conte, and Kieffer pears, but in all other varieties which they examined malic acid predominated, and that Thompson, Firman, and Whittier (28) found that in Bartlett pears the sugar present in greatest amount was fructose, together with some sucrose and relatively little glucose.

Other investigators have made observations with a view to determining the best time for picking and the best temperature for storage. It has been found that the optimum temperature for storage depends upon the degree of maturity of the fruit at the time of picking. In general, storage at 32° F. gives a longer storage life than higher temperatures, but it has been shown by Magness (19), Stubenrauch and Ramsey (26), and Overholser and Latimer (23) that fruit picked when very immature keeps better at 36° F. than at 32° F., wilting being very pronounced at the lower temperature. These investigators have also shown that immature pears have a shorter storage life than fruit picked a little later. Shamel (25) has pointed out that at a relatively high temperature (about 83° F.) the ripening of Bartlett pears is retarded, and Overholser and Taylor (24) have confirmed

[1] The fruit was kindly stored by Dr. C. West at the Low Temperature Research Station, Cambridge. It was hoped to repeat the estimations on pears stored at 4° C., but a store at this temperature was not available in 1926, so storage at 5° was substituted.

this, and shown that at temperatures between 87° and 110° F. ripening is slower than at 75° and 85° F. This effect was attributed by Shamel to the high humidity of his store, but Overholser and Taylor have shown that it is more probably due to a retardation of the enzymic activity of the pear when the optimum temperature for this activity is exceeded. Storage at temperatures above 110° F. caused very rapid break-down. It was shown that in the case of apples (Yellow Newtowns) the rate of ripening steadily increased with temperature from 32° F. until at 85° F. the fruit was completely browned.

Magness (19) gives a review of previous work on the chemical composition and storage of pears, and an account of his own work on Bartlett pears from different localities. He studied the effect of the state of maturity at picking on the chemical composition during ripening in store at different temperatures. There was an increase in total sugars (calculated as percentages of the fresh weight of the pulp at time of analysis) from immaturity to the close of the picking season, the increase during the earlier part of the season being mainly due to accumulation of reducing sugars, and in the latter part to sucrose. The acid content of the fruit was higher in the early pickings than in the last ones in the Californian fruit, but the reverse was the case in fruit from Oregon and Washington. The content of total solids was lowest at the beginning of the commercial picking season, and increased with the increase in sugars, while reducing material which was alcohol-insoluble, and acid hydrolysable, decreased steadily from the beginning to the end of the season.

After ripening in store, the fruit kept at 30° F. had a higher sugar content than that kept at 40° F. but lower than that ripened at 70° F. The acidity in general decreased in store, but in the case of fruit picked fairly mature and stored at 70° F. the acidity increased on ripening. A small decrease in dry weight was found in some cases, and the alcohol-insoluble, acid-hydrolysable substances, including starch (where present), hemicelluloses, galactans, and the pectic compounds, decreased during storage. To account for the high sugar content of the pears ripened at 70° F., Magness suggests that either less sugar may be respired than at lower temperatures, or more non-reducing substances may produce reducing substances as ripening proceeds. It is stated that all the storage chambers had a comparatively high humidity, but the actual figures are not given, and it seems very probable that the high sugar content of the fruit ripened at 70° F., as well as the increase of acidity on ripening, may have been due to a larger loss of water by transpiration than at the lower temperatures. This has not been taken into account in calculating the values, and the results obtained in the present investigation show the necessity for making a correction for the effect of water-loss when estimating changes in the amounts of the constituents of the fruit.

Luthra (18) studied the effect of dry and moist air on the rate of respiration and break-down of ripe Carteret and Conference pears, and found that in dry air the rate of break-down was greater than in moist air in the Conference pears, and was accompanied by increased transpiration, and consequently by an increase in the specific gravity of the juice. There was a decrease in the rate of respiration towards the end of the experiments which coincided with the gradual softening and break-down of the fruit.

Recent investigations by Harley and Fisher (13) have shown that acetaldehyde is a normal product of the metabolism of Bartlett pears, and the amount present increases during the period of ripening. Pears suffering from scald and break-down have been shown to have a much higher content of acetaldehyde than those of the same batch which remained sound, and the content in the broken-down tissue was found to be greater than in sound tissue from the same pears. These investigators have suggested that the browning of the tissues in the broken-down fruit is due to the toxicity of acetaldehyde, and that after a certain concentration of this substance is reached, browning and break-down take place.

III. Scheme of Analysis and Methods of Estimation.

In the preliminary investigation carried out on the pears stored at 12° C. and 1° C. in 1924, the following estimations were made at frequent intervals:

(*a*) total pectic content (protopectin [1] + pectin);
(*b*) pectic content of the juice;
(*c*) acidity ,, ,,
(*d*) viscosity ,, ,,

Similar estimations were made on the pears stored at 4° C. in 1925 and at 5° C. and 1° C. in 1926, in addition to the following determinations:

(*e*) hardness of fruit;
(*f*) dry weight;
(*g*) residue after alcoholic extraction;
(*h*) total sugars [2];
(*i*) reducing sugars [2];
(*j*) acidity [2];
(*k*) specific gravity of juice.

[1] The term 'protopectin' has been substituted for 'pectose' used by Carré (6) to denote the water-insoluble, acid-hydrolysable pectin substance which exists in complex combination in the cell-wall, and is the precursor of soluble pectin. It is considered that the term 'pectose' suggests a simple carbohydrate constitution for the substance for which there is no experimental evidence.

[2] The estimations of sugars and acidity in the pears stored at 4° C. were kindly made by Dr. D. I. Evans.

Preparation of Sample for Analysis. The pears were weighed individually before being stored, and again when the estimations were made, so that the change in concentration brought about by the loss of weight during storage could be taken into account when calculating changes in the amounts of the constituents (14).

The sample for analysis consisted of ten pears, which were peeled, cored, and cut very finely by hand and thoroughly mixed. A portion of the pulp was frozen in metal cylinders and the juice expressed, as described by Haynes and Judd (15).

The estimations of total pectic content, soluble pectin, and alcohol-insoluble materials were made on fresh pulp, and the rest of the estimations on freshly expressed juice.

Methods of Analysis.

Estimation of the Pectic Content. The method of extraction and estimation of the pectic compounds was that described by Carré (6). For the combined estimation of the pectin and protopectin 50 grm. of the pulp were extracted for five minutes with boiling water, the extract filtered off, and the pulp re-extracted by successive three-hour periods of boiling with N/75 HCl, until no precipitate of calcium pectate could be obtained from the extract. The pectic content of the extracts was estimated by the calcium pectate method (7).

In the case of the soluble pectin, 50 grm. of pulp were frozen and extracted with cold water as described by Carré. It was, however, found necessary, in order to remove suspended material, to centrifugalize the extract before precipitating as calcium pectate. Carré recommends filtration, but in the case of ripe pears this was found inadequate. Table I shows the magnitude of the error which may be introduced into this estimation by suspended material when the fruit is soft and ripe. The accuracy of the result obtained on centrifugalized extracts was tested by a comparison with the result obtained by purifying by preliminary precipitation with an N/10 solution of HCl in alcohol (Emmett and Carré (11)). It was also tested by comparing the soluble pectin content of the sample, estimated directly, with the value obtained from the difference between the total pectic content and the protopectin content, the latter being estimated by boiling the residue from the soluble pectin extraction with N/75 HCl. In addition, a solution of pure pectin was made up and estimated before and after centrifugalizing to make quite sure that no pectin was thrown out of solution by this treatment.

It will be seen from Table I that there is good agreement between the percentages of soluble pectin estimated on extracts purified by centrifugalization and by preliminary precipitation with acidified alcohol. There is also fairly good agreement between the estimations made directly and

by difference. Results obtained by the use of alcohol are slightly lower than those obtained by direct precipitation as calcium salt. This has always been found to be the case, and is probably due to the extreme dilution of the extract, resulting in slightly incomplete precipitation by acid alcohol, and in part to a slight contamination of the calcium pectate precipitate by other substances present in the extract. In any case, the results show quite clearly that the estimation of soluble pectin on uncentrifugalized extract may give a result more than 100 per cent. too high in the case of soft ripe tissues.

TABLE I.

Effect of Suspended Material on Soluble Pectin Estimation.

Sample.	% of Soluble Pectin. (Direct Estimation)				% Protopectin + pectin.	% of Protopectin.	% of Soluble Pectin (estimated by difference.)
	Uncentrifugalized. Calcium Pectate Method.		*Centrifugalized.* Calcium Pectate Method.				
	Without Acid-alcohol.	With Acid-alcohol.	Without Acid-alcohol.	With Acid-alcohol.			
(1) Rather over-ripe.	0·56	0·23	0·27	—	0·49	—	—
(2) Over-ripe	0·56	0·19	0·22	0·19	0·47	0·28	0·19
(3) Solution of pure pectin.	0·10	—	0·10	—	—	—	—

Pectic Compounds of the Middle Lamella. Carré (6) has found it possible to extract a third fraction of pectic material from apples by boiling the residue after the extraction of pectin and protopectin, with N/75 NaOH for successive periods of thirty minutes.

She has suggested that this fraction consists of pectic material from the middle lamella. The method has not been established as quantitative, and although small amounts of a substance precipitable in the same way as pectin are extracted by alkali from the pears, the results given in Table II show that this treatment must result in the destruction of a considerable amount of pectin.

TABLE II.

Showing the Effect of Boiling a Solution of Apple Pectin with NaOH.

	Weight of Calcium Pectate from 25 c.c. of Solution.			
	Unboiled.	Boiled 15 mins. with N/75 NaOH.	Boiled 30 mins. with N/100 NaOH.	% Destroyed.
I.	0·0360	0·0294	—	19·0
	0·0363	0·0292	—	—
II.	0·0310	—	0·0258	17·6
	0·0314	—	0·0256	—

Attempts to extract the pectic substance of the middle lamella of pears by shaking the tissue with N/75 NaOH in the cold resulted in the extraction of exceedingly small quantities of the substance, and both in this case and in the case of extracts made by boiling, the calcium pectate precipitate obtained was sticky and difficult to wash, also it was found that duplicate extractions of similar samples of tissue seldom gave concordant results.

The results of estimations by this method made at intervals during the storage season are therefore not included here, as they were apparently of no value, even as a relative measure of the amount of pectic substance in the middle lamella.

The pectin of the juice was estimated by direct precipitation as calcium pectate.

The viscosity of the juice was determined by the Ostwald method at 25° C., the gelling action of the pectase being inhibited by acidifying with HCl to make the resulting concentration N/25.

Hardness of Fruit. The hardness was determined by pressure, using a machine similar to that described by Murneek (20). This machine registers the hardness of pears in terms of the pressure required to force a metal plunger a given distance into the fruit, the hardness being directly proportional to the applied pressure. It has been found that the pressure varies according to the thickness of the block of pulp tested, and a standard thickness of three-quarters of an inch has been used in all the determinations.

Alcohol-insoluble Residue. To obtain a measure of the total cell-wall material of the fruit, 30 grm. of pulp were extracted with 95 per cent. alcohol, and the residue dried, as described by Archbold (1). This determination merely gives the weight of the dry material of the cell-wall, and is not an estimate of the living cell-wall holding an unknown quantity of water of imbibition. The soluble pectin is included in this value, since it is precipitated from the juice by alcohol, and the stone cells are also included.

Stone Cells. Dorée and Barton-Wright (9) have shown that the stone cells of the pear consist of 80 per cent. lignocellulose. A few rough estimations of the amounts in which they are present have been made, by dissolving the cellulose from the alcohol-extracted pulp, with Schweitzer's reagent, the residue of lignified stone cells then being washed, first with acidified water and finally with boiling water, and dried at 100° C. and weighed.

Dry Weight. Archbold has very recently (2) shown that the dry weight of apple juices may be found to within 2 per cent. of the correct value by absorbing the juice in pumice and drying *in vacuo* at room temperature. The method was worked out while the present investigation was proceeding, and it has therefore been possible to apply it to the last set of pears (1926–7). In the earlier estimations of dry weight a high temperature was employed; this has been found to be inaccurate, and the results

are therefore omitted. That the method of drying at room temperature, although worked out especially for apple juices, is applicable to pears is shown by the fact that the values obtained for the dry weights of the pear juices agree reasonably well with the values for total solid content calculated from the specific gravity measurements, by the use of the empirical formula

$$W = \frac{1000\ (\sigma - 1)}{3{\cdot}86}$$

when

W = total solids in 100 c.c. of juice and

σ = specific gravity.

An example of this agreement is given in Table III, and the calculated values for the whole series in sets stored at 4°, 5°, and 1° C. are given in Tables VII, VIII, IX.

Archbold has shown that a measure of the total dry weight of apple tissue may be obtained from the sum of the alcohol-extracted residue (i. e. cell-wall material with the addition of soluble pectin) and dry weight of the juice per 100 grm. of pulp. The results given in Table III show that this is also the case for pear tissue, very good agreement being obtained between the dry weight of the tissue dried *in vacuo* and the sum of the alcohol-insoluble residue and 'dry weight' of the juice.

TABLE III.

Date.	Dry Weight of Juice—W.	Dry Weight of Juice calc. from Specific Gravity.	Alcohol-extracted Residue—R.	W + R.	Dry Weight of Pulp.
10. 2. 27	10·43	10·39	2·26	12·69	12·74
4. 4. 27	10·44	10·30	2·18	12·62	12·50

Sugars. For the estimation of the sugars (total and reducing sugar, fructose and glucose), the methods described by Evans (12) were used. Since the estimations were made, it has been shown by Haynes and Archbold (14) that the results obtained in this way are 2–3 per cent. below the true values.

Acidity was determined by titrating 20 c.c. of the juice with N/10 NaOH, using ten drops of phenol-phthalein as indicator. As the end point is by no means definite, the titration was made to a standard colour (3). The acid was calculated as malic acid. It has already been remarked that the acids contained in the various varieties of pear which have been analysed are chiefly citric and malic. It is not known which acid predominates in the Conference pears used in this investigation, but the error introduced by calculating citric acid as malic acid is practically negligible when the total amount of acid present is as small as it is in this case.

Expression of Results. The values obtained are expressed as per-

centages of the fresh weight of the pulp when placed in store, the loss of weight of the fruit during storage being taken into account (14).

Sampling Error. In order to ascertain the variations which may occur in the chemical composition of pears of the same set, estimations of some of the constituents of ten individual pears have been made, and the probable error of the mean of ten results has been calculated. The results are given in Tables IV and V.

TABLE IV.

Estimations of Variations in Total Pectic Content.

Pears stored at 1° (Season 1924).

	% Pectin + Protopectin.
1	0·51
2	0·50
3	0·49
4	0·47
5	0·48
6	0·50
7	0·49
8	0·53
9	0·51
Mean	0·50 ± 0·0048 (i.e. ± 1 %).

TABLE V.

Estimation of Variations in Residue, Specific Gravity, and Acidity.

Pears stored at 1° (Season 1926).

No.	Loss of Weight in 152 days.	Acidity.	Specific Gravity.	Residue.
1	25·9	0·104	1·061	2·18
2	25·0	0·101	1·062	2·41
3	23·8	0·042	1·058	2·52
4	26·7	0·098	1·067	2·20
5	28·9	0·048	1·064	1·82
6	29·1	0·069	1·062	2·00
7	30·9	0·115	1·059	2·16
8	27·6	0·062	1·057	2·14
9	43·1	0·080	1·065	2·10
10	30·3	0·094	1·061	2·07
Mean	29·1 ± 1·15 i.e. ± 3·97 %	0·081 ± 0·005 i.e. ± 6·17 %	1·062 ± 0·007 i.e. ± 0·65 %	2·16 ± 0·04 i.e. ± 1·85 %

It will be seen that the fruit stored in 1926 has a very large sampling error, and the large scatter of the points on the graphs showing changes in total sugar, dry weight of juice, and especially acidity (Fig. 1), may certainly be attributed to this. These graphs, in fact, serve to emphasize the need for the use of as large samples as possible in work of this nature, a sample of ten pears being far too small where the individual variations are so large.

Table VI.

Analysis of Conference Pears (1924).

Analyses calculated as percentages of fresh weight at time of analysis.

	Stored at 12° C. (Set A 12°.)				Stored at 1° C. (Set A 1°).			
Days in Store.	Pectin + Protopectin.	Pectin in Juice (grm./100 c.c).	Viscosity of Juice.	Acidity of Juice. (grm./100 c.c.).	Pectin + Protopectin.	Pectin in Juice (grm./100 c.c.).	Viscosity of Juice.	Acidity of Juice (grm./100 c.c.)
0	0·51	0·08	2·258	0·086	0·51	0·08	2·258	0·086
12	0·50	0·32	4·220	0·066	0·50	0·07	2·199	0·070
19	0·41	0·28	2·360	0·053	0·52	0·07	2·182	0·079
27	0·26	0·21	1·742	0·063	—	—	—	—
39	—	—	—	—	0·50	0·06	2·148	0·082
60	—	—	—	—	0·54	0·05	1·979	0·085
67	—	—	—	—	—	0·08	2·300	—
124	—	—	—	—	0·55	0·11	3·315	0·073
173	—	—	—	—	0·57	0·11	2·732	—

Table VII.

Analysis of Conference Pears stored at 4° C. (1925). (Set B 4°).

Analyses calculated as percentages of original fresh weight of pulp.

Days in Store.	% Loss of Weight.	Pectin + Proto-pectin.	Soluble Pectin	Proto-pectin.	Alcohol-insoluble Residue.	Specific Gravity.	Total Solids in Juice (from Specific Gravity).	Acidity.	Total Sugar.	Reducing Sugar.	Cane Sugar.	Pectin in Juice (grm./100 c.c.)	Viscosity of Juice.
0	0	0·63	0·27	0·36	—	1·054	12·95	—	—	—	—	0·26	5·455
15	1·7	0·60	0·28	0·32	—	—	—	0·092	9·09	6·77	2·32	—	—
29	3·7	0·56	0·25	0·31	2·36	1·053	12·24	0·085	8·73	6·53	2·20	0·26	3·025
43	6·3	0·48	0·22	0·26	2·31	1·054	12·12	0·089	8·85	6·79	2·06	0·23	2·842
57	9·1	0·38	0·20	0·18	2·12	1·053	11·57	0·067	8·81	7·08	1·73	0·26	2·391
71	12·0	0·43	0·23	0·20	2·22	1·051	10·79	0·065	8·63	6·86	1·77	0·16	2·041
99	17·7	0·39	0·19	0·20	2·18	1·054	10·63	0·048	7·99	6·55	1·44	0·17	2·152
113	18·5	0·36	0·15	0·21	2·19	1·058	11·26	0·054	8·19	7·22	0·97	0·13	1·842

TABLE VIII.

Analysis of Conference Pears stored at 5° C. (1926). (Set C 5°.)

Analyses calculated as percentages of original fresh weight of pulp.

Days in Store.	% Loss of Weight.	Pectin + Proto-pectin.	Soluble Pectin.	Proto-pectin.	Alcohol-Insoluble Residue.	'Dry Weight' of Juice.	Specific Gravity.	Total Solids in Juice (from Specific Gravity).	Acidity.	Total Sugar.	Reducing Sugar.	Cane Sugar.	Fructose (F).	Glucose (G)	F/G.
0	0	0·62	0·13	0·49	2·78	11·35	1·046	11·08	0·071	8·16	5·91	2·25	4·78	1·45	3·3
8	2·0	0·57	0·20	0·37	2·45	11·18	1·047	11·11	0·055	8·03	6·05	1·98	4·87	1·35	3·6
20	4·0	0·62	0·42	0·20	2·31	11·68	1·049	11·34	0·085	8·60	6·19	2·41	5·08	1·34	3·8
28	6·6	0·59	0·31	0·28	2·40	10·76	1·048	10·80	0·092	7·73	6·19	1·54	4·94	1·56	3·2
35	8·0	0·54	0·46	0·08	2·23	10·79	1·049	10·87	0·093	7·53	6·20	1·33	4·66	1·67	2·8
42	7·9	0·42	0·37	0·05	2·13	11·55	1·050	11·10	0·087	8·35	6·57	1·78	5·35	1·39	3·9
56	8·6	0·37	0·34	0·03	2·27	11·38	1·050	10·99	0·080	7·91	6·72	1·19	5·34	1·57	3·4
63	10·3	0·33	0·30	0·03	2·22	10·82	1·048	10·39	0·089	7·94	6·76	1·18	5·33	1·54	3·4
76	11·6	0·31	0·30	0·01	2·14	10·78	1·049	10·44	0·072	7·81	6·79	1·02	5·42	1·48	3·7

TABLE IX.

Analysis of Conference Pears stored at 1° C. (1926). (Set C 1°.)

Analyses calculated as percentages of original fresh weight of pulp.

Days in Store.	% Loss of Weight.	Pectin + Proto-pectin.	Soluble Pectin.	Proto-pectin	Alcohol-Insoluble Residue.	'Dry Weight' of Juice.	Specific Gravity.	Total Solids in Juice (from Specific Gravity).	Acidity.	Total Sugar.	Reducing Sugar.	Cane Sugar.	Fructose (F).	Glucose (G).	F/G.
0	0	0·62	0·13	0·49	2·78	11·35	1·046	11·08	0·071	8·16	5·91	2·25	4·78	1·45	3·3
8	2·2	0·57	0·18	0·39	2·56	11·44	1·047	11·08	0·079	8·17	5·82	2·35	4·65	1·19	3·9
28	7·7	0·57	0·13	0·44	2·45	—	—	—	0·047	7·82	6·00	1·82	4·67	1·53	3·1
35	7·6	0·55	0·18	0·37	2·13	10·85	1·049	10·92	0·052	7·77	6·17	1·60	4·88	1·48	3·3
42	10·1	0·58	0·18	0·40	2·11	—	—	—	0·067	8·20	6·38	1·82	4·85	1·55	3·1
56	13·2	0·56	0·18	0·38	2·28	10·97	1·051	10·62	0·102	7·91	6·46	1·45	4·94	1·63	3·0
76	17·9	0·51	0·20	0·31	2·22	—	—	—	0·090	7·57	6·35	1·22	4·75	1·74	2·7
111	22·1	0·48	0·20	0·28	2·15	11·02	1·059	10·93	0·063	7·92	6·67	1·25	5·04	1·71	2·9
140	26·8	0·45	0·22	0·23	2·26	10·43	1·060	10·40	0·054	7·41	6·16	1·25	4·71	1·58	3·0
152	29·1	—	—	—	2·17	—	1·062	10·39	0·081	—	—	—	—	—	—
194	33·6	0·45	0·20	0·25	2·18	10·44	1·066	10·30	0·080	7·60	6·34	1·26	4·86	1·57	3·1

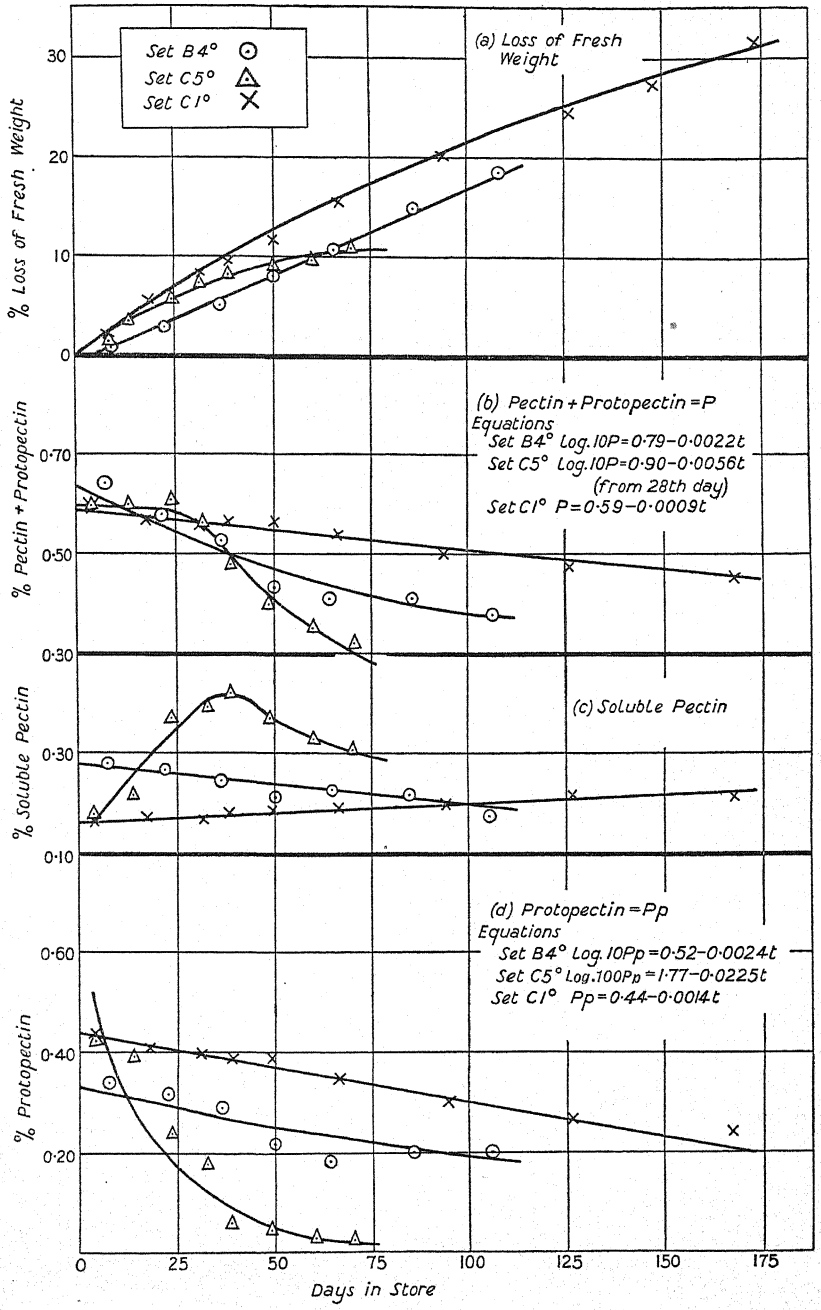


FIG. 1 (*a*, *b*, *c*, *d*). Changes in fresh weight and pectic content of Conference pears stored at different temperatures.

IV. Rate of Change during Storage.

In the discussion of these changes the different sets of pears are numbered as follows:

Set A 12° stored at 12° C. in Sept. 1924
„ A 1° „ 1° C. „ „
„ B 4° „ 4° C. „ 1925
„ C 5° „ 5° C. „ 1926
„ C 1° „ 1° C. „ „

The results of the analyses are given in Tables VI, VII, VIII, IX, and shown graphically in Fig. 1. In this figure an attempt has been made to reduce the scatter due to sampling error by averaging two adjacent points. Thus, if *a*, *b*, *c*, *d* are a number of points along a curve, the mean of *a* and *b* would be taken as the first point, that of *b* and *c* as the second, of *c* and *d* as the third, and so on. Except in the case of loss of weight, soluble pectin, and reducing sugar, curves of closest fit have been calculated to the experimental points. Table X gives some observations on the general behaviour of the different sets in store.

Table X.

Effect of Temperature of Store on Ripening.

Set.	Temp. of Store.	Humidity of Store.	Condition when placed in Store.	Time taken to Ripen.	Behaviour of Starch.	Remarks.
A 12°	12° C.	—	Hard and unripe.	About ten to twelve days.	—	Observations discontinued after three weeks owing to complete break-down of fruit.
A 1°	1° C.	—	Hard and unripe.	Remained green, did not ripen.	—	Fruit wilted—supply exhausted after six months.
B 4°	4° C.	—	Almost ripe.	Within two weeks.	None present when placed in store.	Browning of pulp apparent after six weeks. Observations discontinued after four months owing to complete break-down.
C 5°	5° C.	80%	Very hard and unripe.	Three weeks.	Disappeared in eight days.	Browning of pulp apparent after one month. Observations discontinued after ten weeks owing to fungal attack.
C 1°	1° C.	80%	Very hard and unripe.	Did not ripen. Very slight softening after three months.	Disappeared in three to four weeks.	Wilting very pronounced. Observations discontinued after six months, owing to fungal attack.

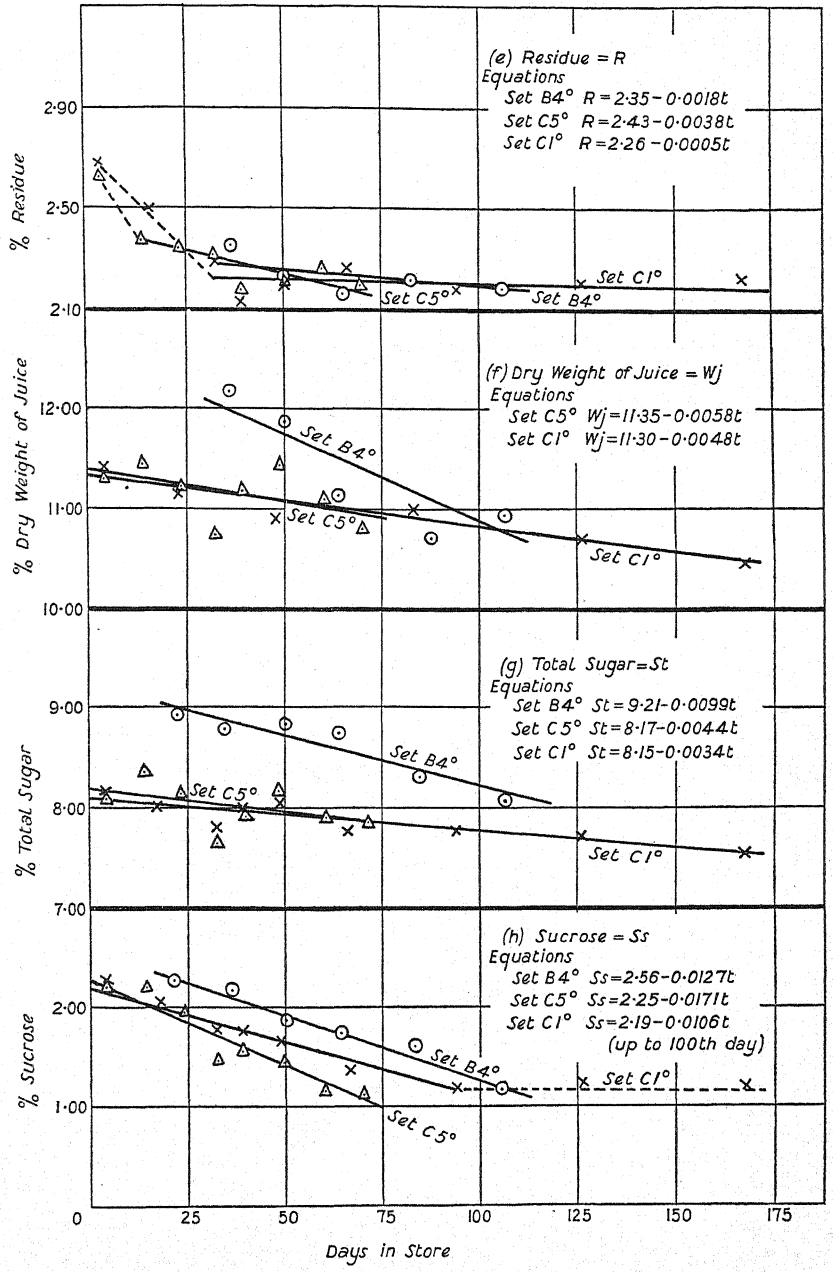


FIG. 1 (*e*, *f*, *g*, *h*). Changes in residue, 'dry weight' of juice, total sugar and sucrose content of Conference pears.

Loss of Weight during Storage. The fruit lost weight during storage as a result both of transpiration and respiration. Under the conditions of these experiments the latter process accounted for less than 10 per cent. of the total loss of weight. The observed rate of loss of weight is therefore determined almost entirely by the rate of transpiration.

The rate of loss of weight of these sets during the greater part of the storage season was constant, in sets C 5° and C 1° it fell off slightly towards the end of the season. Set C 1° lost weight surprisingly rapidly, more rapidly, in fact, than C 5°, although the humidity of both stores was the same. The storage chambers were, however, markedly different in size and arrangement, and it is therefore possible that some factor influencing transpiration may have been introduced in this way. It is also probable that the difference is due to a temperature effect on the lenticels. Magness (19) records an experiment in which a freshly picked immature Bartlett pear placed in methylene blue was compared with a ripe pear similarly treated. In the case of the immature fruit, the dye penetrated the skin, but no penetration took place in the ripe pear, which was found to have a layer of suberised tissue in the lenticels. Magness also noted that pears picked when immature wilted more in store than those picked at a later stage, and attributed this to the formation of the suberised layer in the lenticels on ripening. Overholser and Latimer (23) in discussing this question, have pointed out that immature pears lack the cutin layer of the skin, which develops on maturity and is only slightly permeable. In the present investigation, sets C 1° and C 5° were very unripe when placed in store. At 5° C. the fruit ripened in three weeks, and the ordinary changes accompanying maturity presumably took place, while at 1° C. only slight signs of ripening were apparent after six months, and, therefore, the suberised layer in the lenticels and the cutin covering of the skin may never have developed.

Changes in Concentration of the Constituents. In the tables (VII, VIII, IX) and Fig. 1, and the subsequent discussion of the rate of change of the various constituents, the values are given in terms of the fresh weight of the pulp when placed in store, as already described. These results show a decrease in the total amount of solid material in the pear, owing to the consumption of respirable substances. The loss of water due to transpiration, however, brings about an actual increase in the concentration of solids in the pulp, in spite of the losses due to respiration. Thus, there is in each set of pears an increase in the total concentration of sugars and solid material (dry weight) and in the specific gravity of the juice. The inversion of sucrose and the break-down of the pectic compounds, however, proceed proportionately more rapidly than the loss of water so that their concentration decreases.

This concentrating effect of transpiration must influence the rates at which the metabolic processes takes place. A decrease in the concentra-

tion of a constituent undergoing break-down would be expected to decrease the rate of break-down according to the law of mass action, and the effect of transpiration in keeping up, and actually increasing the concentration of solids in the fruit should, therefore, be to prevent this decline in rate. This

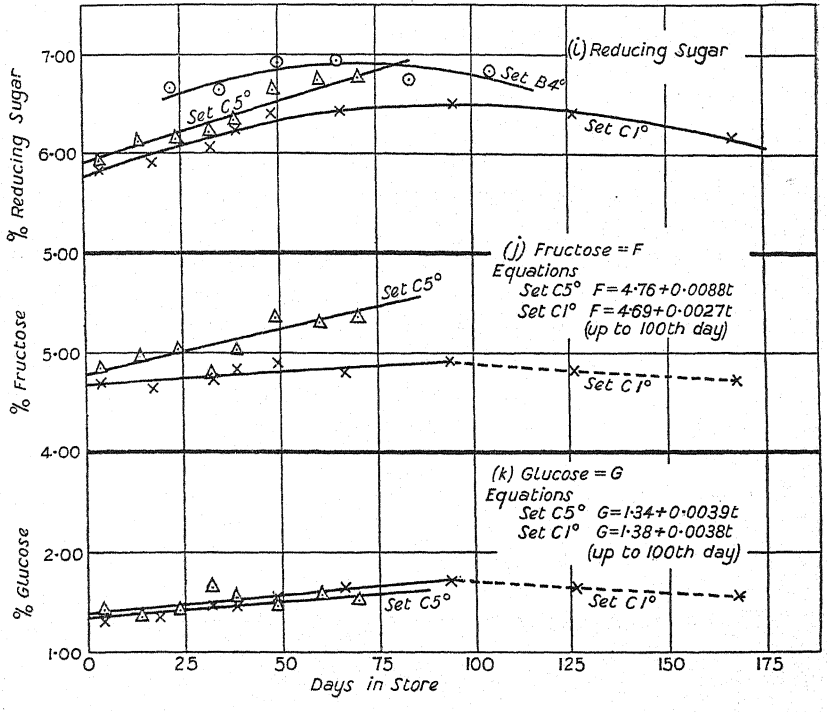


FIG. 1 (*i*, *j*, *k*). Changes in content of reducing sugars in Conference pears.

has been found to be the case for total sugar and total solid content in the sets of pears stored at 4° and 5° C.

Changes in Pectic Compounds. The marked differences in rate of ripening at the different temperatures were accompanied by interesting differences in the changes in pectic content of the fruit. (*a*) In the preliminary set A 12° (Table VI) there was a large development of soluble pectin[1] at the expense of protopectin during the first fortnight in store, when the fruit was ripening, but no decrease in the total amount of pectic substances present. During the last fortnight the rapid browning and breakdown of the fruit were accompanied by the disappearance of 50 per cent. of the pectic material.

In set A 1° the pectic changes were very small, the total pectic content showing a slight increase when calculated on the fresh weight of the pulp

[1] No soluble pectin estimations were made on this set, but the pectin content of the juice gives a satisfactory measure of the soluble pectin for comparative purposes.

at the time of analysis. No data for the loss of weight were available for this set, however, but the data obtained at the same temperature in 1926 indicate that were a correction made for this, a small decrease in pectic content would be shown. There was a very small apparent development of soluble pectin, which was almost certainly also due to the increased concentration caused by the loss of weight.

(*b*) Set B 4° (Table VII) showed a fairly steady decrease in total pectic content during the whole storage life, with a slight retardation of rate of loss towards the end. The soluble pectin was at its maximum when the fruit was put into store, and did not show such a rapid rate of loss as the protopectin since the former was augmented by the break-down of the latter. The production of pectin from protopectin, however, became very slow when the percentage of the latter was reduced to about 0·20 (i. e. after seven weeks). That the rate of conversion of protopectin to pectin, as well as the further break-down of the pectic compounds, most probably takes place according to the exponential law, is shown by the results obtained at 5° in the following season. In the set B 4°, however, the departure from a constant rate of break-down is exceedingly small in the case of the total pectic content.

(*c*) Set C 1° behaved in a similar fashion to set A 1°. There was a definite slow decrease in total pectic content, beginning after about six weeks storage, and very much smaller than that observed at higher temperatures. Very little development of soluble pectin took place, the rate of hydrolysis of protopectin being scarcely more than enough to balance the amount of pectin which disappeared during storage.

(*d*) During the first weeks in store, set C 5° (Table VIII) showed no decrease in the total pectic content, but a rapid development of soluble pectin took place at the expense of protopectin. When the fruit was becoming over-ripe, rapid break-down of the pectic compounds set in, the conversion of protopectin into pectin continuing at a decreasing rate, until the protopectin had almost disappeared. This complete disappearance of protopectin had not been observed in any of the other sets of pears, or in apples examined by the author, but Carré (6) has noted complete disappearance of protopectin from apples in advanced stages of break-down. The results obtained provide evidence that both the conversion of protopectin to soluble pectin, and the further break-down of pectin, take place according to the exponential law, the rate of chemical change being dependent upon the amount present of the substance which is breaking down. In Fig. 1 (*b* and *d*) the curves showing the changes in total pectic content and protopectin are the logarithmic curves calculated to fit the experimental points. It will be seen that the scatter of the points round the curves is very small. The fact that there is no decrease in the total pectic content until the period of full ripeness is past is difficult to explain.

The same thing occurs in the case of apples, and Carré suggests that the acidity is the factor which inhibits the enzyme causing break-down, this inhibition being removed when the acidity has declined below a certain limit. It seems improbable that this explanation holds in the case of the pears examined, since no measurable decline in the very small content of acid was observed. This point will be discussed later.

Viscosity and Pectic Content of the Juice. Comparisons of the viscosity and pectic content of the juice were made in sets A and B, in order to ascertain to what extent viscosity was an index of the state of maturity of the fruit, and whether there was evidence of any chemical or physical change in the soluble pectic compounds during the storage life.

Some preliminary experiments were made with artificially prepared solutions containing apple pectin and cane sugar in varying amounts to determine the effect on the viscosity of the juice of changes in the sugar concentration. These experiments showed that changes in the total sugar content of the solutions, of the magnitude of those which occur during storage in the juices investigated, i. e. differences of less than 1·5 grm., could have very little effect upon the viscosity. The acidity changes in the pear juices are very small, and unless other colloids are present changes in the viscosity of the juice will be determined by changes in the pectic content.

Reference to Fig. 2 shows that in Set A 1°, which did not ripen, there is close parallelism between the curves showing changes in the viscosity and pectic content of the juice respectively. In the case of Set A 12° a large development of soluble pectin in the first fortnight was accompanied by a considerable increase in viscosity, but after this point, when the fruit was becoming over-ripe, there was a much more marked decrease of viscosity than in pectic content, the former being lower at the end of the storage life than at the beginning, although the final pectic content was considerably higher than at the time of storing. In Set B 4° a similar result was obtained. The pectic content of the juice was at its maximum when the fruit was placed in store, and remained practically constant for about eight weeks, during which period there was a rapid fall in viscosity. After this time there was close parallelism between the changes in viscosity and pectic content. It therefore appears that the same relationship between viscosity and pectic content does not hold throughout the whole of the storage life. It is possible that the marked fall in viscosity after the period of full ripeness is past is due to the disappearance of some other colloid, which is present in the juice in the earlier stages. On the other hand, the pectin itself may undergo either physical or chemical change. Some authorities (6) consider that demethoxylation of the neutral pectin, with the formation of pectinic acids, takes place when the fruit becomes over-ripe, and Sucharipa (27) has found that the viscosity of solutions of pectic substances decreased with decreasing methoxyl content. The fall in viscosity of the

juice may, therefore, be due to demethoxylation of the pectin. It must be borne in mind that such a change in the pectic compounds would have no effect on the pectic content estimated as calcium pectate, since both neutral pectin and pectinic acid molecules would yield pectic acid on hydrolysis.

Relation between Pectic Content and Hardness. The determination of hardness was only made in the case of the pears stored at 4° in 1925.

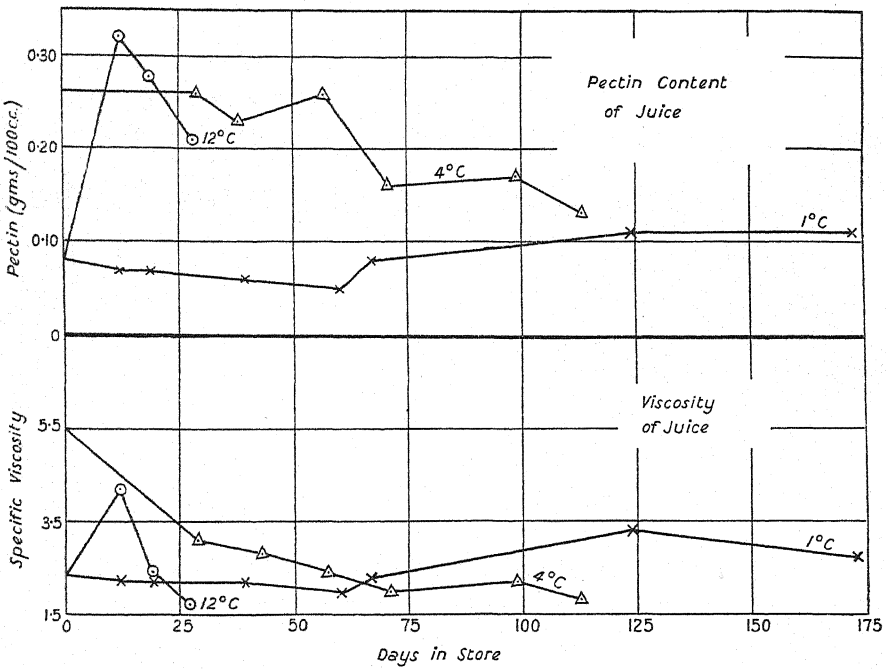


FIG. 2. Comparison between pectic content and viscosity of juice of Conference pears stored at different temperatures.

As has already been noted, these were practically ripe when placed in store, and rapidly became very soft. Although the turgor of the cells must also be a determining factor, it is the usually accepted view that the pectic compounds are chiefly responsible for changes in the hardness of the fruit, the conversion of insoluble protopectin into soluble pectin causing softening of the tissues. This view is confirmed by the results shown in Table II and Fig. 3. It seems reasonable to suppose that the relative hardness of different samples of different pectic content will be determined by the fraction of the total pectic content which is present as protopectin, rather than by the actual amount of protopectin present. In Fig. 3, therefore, the hardness is plotted against the percentage of the total pectic content which is present as protopectin.

It will be seen that the hardness decreases as this percentage decreases, but the rate of softening decreases as the content of protopectin becomes

smaller. The last determinations were made when the fruit was very soft and juicy, and the cells were tending to separate from each other. When

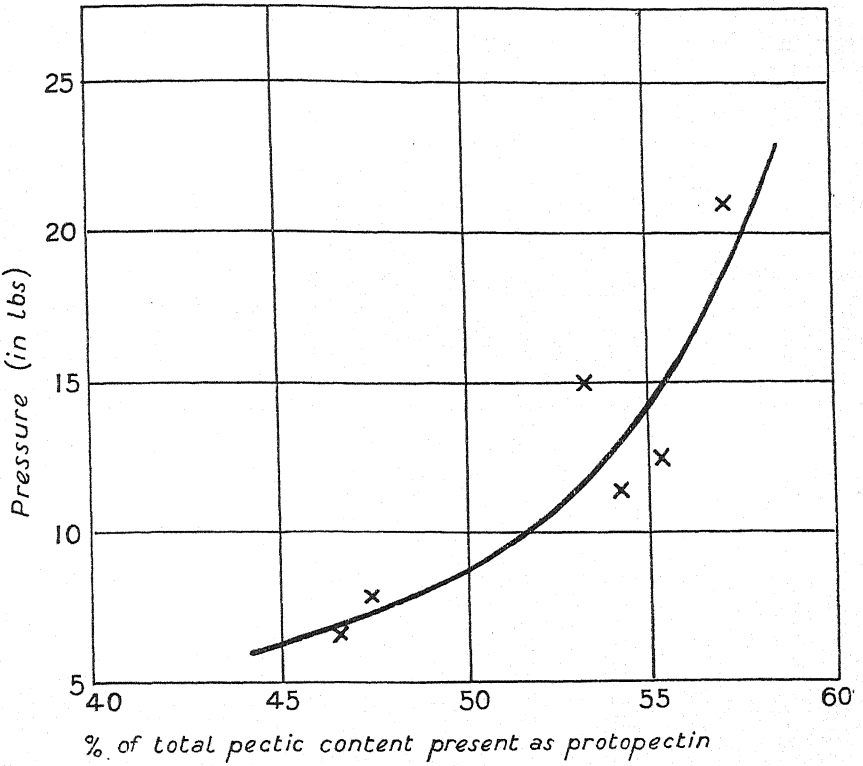


FIG. 3. The relation between content of protopectin and hardness of Conference pears stored at 40° C. (set B 4°).

this stage has been reached and the cell framework is no longer complete, a decrease in the fraction of the pectic content present as protopectin has less effect on the hardness of the fruit than at the beginning of the season.

TABLE XI.

Comparison of Hardness and Soluble Pectic Content.

Days in Store.	Hardness (Pressure in lb. for one Pear (Mean of ten Determinations)).	Percentage of Total Pectic Content present as Protopectin.
0	20·80	57·14
15	15·00	53·33
29	12·50	55·35
43	11·25	54·16
57	7·70	47·37
71	6·50	46·51

Further evidence that the development of soluble pectin is the chief factor concerned in the softening of fruit is afforded by a comparison of the

results obtained for a set of apples at 8° C. in 'gas store', i. e. in an atmosphere containing 9 per cent. of CO_2 and 11 per cent. of oxygen with a control set at the same temperature in ordinary air. In Table XII and Fig. 4 are given the results of estimations of the hardness and pectic content of the juice in these sets.

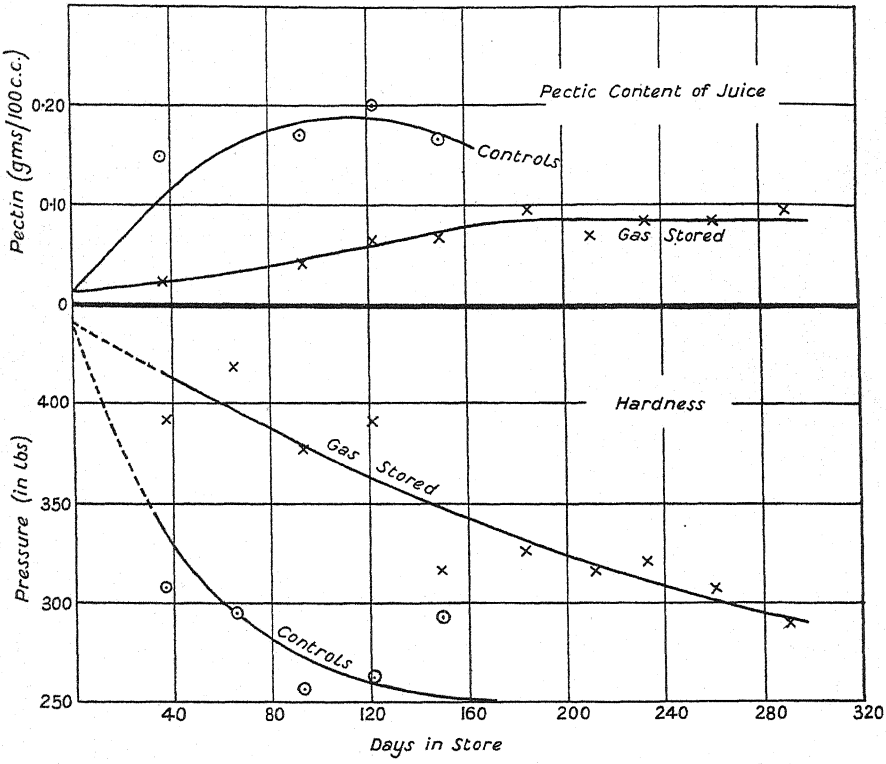


FIG. 4. Development of soluble pectin and softening of Bramley's Seedling apples in (*a*) gas store, and (*b*) ordinary air at the same temperature (8° C.).

Kidd and West (17) have shown that gas storage greatly decreases the respiration rate and increases the storage life of the fruit. It will be seen from the results given here that softening is greatly retarded in gas store, and that this is associated with the retardation of the development of soluble pectin. The fruit stored in ordinary air attained its maximum content of soluble pectin (0·20 per cent.) in 121 days, while in gas store the soluble pectin content reached 0·09 per cent. in 184 days, after which time no further increase took place.

Changes in the coherence of the cell-wall caused by the dissolution of the middle lamella have apparently very little effect on the hardness of the fruit, for it is found that both in apples and pears the rate of softening becomes very much slower after the maximum development of soluble

pectin has occurred, although the breakdown of the middle lamella usually takes place after this point. In the absence of a trustworthy method for the estimation of the middle lamella pectic substances, however, the precise effect on hardness of changes in the middle lamella cannot be ascertained.

TABLE XII.

Comparison of Softening and Development of Soluble Pectin in Bramley's Seedling Apples stored at 8°, (a) *in Air containing* 9 *per cent. of* CO_2 *and* 11 *per cent. of Oxygen,* (b) *in Ordinary Air.*

	Ordinary Air.		Gas Store.	
Days in Store.	Pectin in Juice. (grm./100 c.c.).	Hardness (Mean Reading of Pressure Tests in lb. for twenty Apples).	Pectin in Juice.	Hardness.
1	0·013	—	—	—
37	0·148	307	0·022	391
65	—	295	—	418
93	0·169	256	0·039	378
121	0·200	263	0·064	391
149	0·167	293	0·071	315
184	—	—	0·092	326
211	—	—	0·066	318
233	—	—	0·086	321
261	—	—	0·086	306
290	—	—	0·094	289

Changes in Total Cell-Wall Material. As has already been explained, the residue left after alcoholic extraction of the pulp gives a measure of the cell-wall material, including all the pectic compounds present in the fruit.

(*a*) In set B 4° the residue showed a small decrease, which occurred almost entirely during the first three months of storage.

(*b*) Sets C 5° and C 1° showed an initial fall in residue, due to the disappearance of starch from the tissues. This was followed by a slow constant rate of loss at 5°, while at 1° the very slow decrease in residue continued for about two months, after which time the value remained practically constant.

Changes in Total Sugar and Reducing Sugar. (*a*) The set B 4° showed a fairly steady fall in *total sugar* content over the whole storage period. The *reducing sugars*, however, increased, which indicated that the inversion of the sucrose proceeded at a greater rate than the consumption of sugars in respiration. The inversion of sucrose took place at a practically constant rate.

(*b*) Set C 5° had a very large sampling error, the effect of which was especially marked in the total sugar estimations. The results obtained, however, indicate that there was a fall in *total sugar* content of a similar nature to that observed in set B 4°, but very much slower. A particularly

large increase in *reducing sugars* occurred in this set. The rate of inversion of *sucrose* was very slightly retarded towards the end of the storage period.

(*c*) In set C 1° a slight retardation of the rate of loss of *total sugars* took place in the later months of storage, and there was a definite retardation in the rate of inversion of sucrose. After three months in store inversion ceased, possibly owing to the injurious effect of continued exposure to low temperature. The *reducing sugars* showed a considerable increase during the first part of the storage season, but after the inversion of sucrose ceased they showed a small decrease.

It must be remembered that during the rapid loss of starch, the sugar content of the juice is augmented. In this set, complete disappearance of starch did not take place until three weeks after the fruit was placed in store, and in the case of set C 5° this point was reached after a week in store. The actual consumption of sugars, therefore, during the first week in store, must be greater in both sets than is indicated by the analyses.

Changes in Fructose and Glucose. Determinations of the relative amounts of fructose and glucose are only available for sets C 5° and C 1°. In these sets there was an increase in both fructose and glucose during storage. At 5° C. the increase in fructose was much more marked than that in glucose, whereas at 1° C. the increases were more nearly equal. Since glucose and fructose are produced in equal amounts from the inversion of sucrose, these results suggest that at 5° C. there is a preferential oxidation of glucose, which is not the case at 1° C. In set C 1°, after the inversion of sucrose had ceased, there was a small decrease of both glucose and fructose.

Changes in Acidity. The acid content of Conference pears is very low, and since it was found impossible to obtain an end-point when titrating with NaOH weaker than N/10, the titration readings were small. This fact coupled with the large sampling error (Table V) has made the acid determinations of little value, especially for the season 1926–7. In any case, the amount of acid present is so small (less than 0·1 per cent.), even in the unripe fruit, that it can afford very little material for respiration, although the general trend of the results indicates that there is a decrease in acidity as ripening proceeds. The values for sets A 12° and A 1° are only available in terms of grammes of acid per 100 cc. of juice. In set A 12° the fall from 0·09 per cent. to 0·06 per cent. represents a greater decrease in terms of original fresh weight of pulp, as has already been explained, and the concentration values for set A 1°, which show no decrease in acidity, would certainly give a small decrease if similarly calculated.

Set B 4° showed a definite decrease in acidity, but in the case of the fruit stored in 1926, the very large sampling error made it quite impossible to obtain any estimate of rate of loss of acid either at 1° or 5°.

Changes in Total Solids (dry weight) of Juice. Reference to Tables VIII and IX shows that a fairly good agreement is obtained between the values

TABLE XIII.

Showing Difference between Dry Weight of Juice and Sum of Separately Estimated Constituents in 100 grm. of Tissue.

(W_j = dry weight of juice, S_t = total sugars, A = acidity, P = pectin in juice in 100 grm. of tissue.)

Days in Store.	Set B 4°.			Set C 5°.			Set C 1°.		
	W_j (from Specific Gravity).	S_t+A+P.	Difference $W_j-(S_t+A+P)$.	W_j.	S_t+A+P.	Difference $W_j-(S_t+A+P)$.	W_j.	S_t+A+P.	Difference $W_j-(S_t+A+P)$.
0	—	—	—	11·35	8·36	2·99	—	—	—
8	—	—	—	11·18	8·29	2·89	11·44	8·43	3·01
20	—	—	—	11·68	9·11	2·57	—	—	—
28	12·24	9·08	3·16	10·76	8·13	2·63	—	—	—
35	—	—	—	10·79	8·08	2·71	10·85	8·00	2·85
42	12·12	9·17	2·95	11·55	8·81	2·74	—	—	—
56	11·57	9·14	2·43	11·38	8·33	3·05	10·97	8·19	2·78
63	—	—	—	10·82	8·33	2·49	—	—	—
71	10·79	8·86	1·93	—	—	—	—	—	—
76	—	—	—	10·78	8·18	2·60	—	—	—
99	10·63	8·21	2·42	—	—	—	—	—	—
111	—	—	—	—	—	—	11·02	8·18	2·84
113	11·26	8·37	2·89	—	—	—	—	—	—
140	—	—	—	—	—	—	10·43	7·68	2·75
194	—	—	—	—	—	—	10·44	7·88	2·56

found for the dry weight of the juice by direct estimation, and by calculation from the specific gravity, by the formula already given (p. 276). Since no direct estimations are available for set B 4° the calculated values are plotted in Fig. 1, *f*. It will be seen that in all the sets the changes in 'dry weight' of the juice are of the same type as those in the total sugars, but the rate of loss is slightly greater in every case.

Comparison of the dry weight of the juice with the sum of the separately estimated constituents (sugars, acid and pectic substances) indicates that the juice contains a considerable amount of some other constituent of non-reducing and non-acidic character. In Table XIII such a comparison is made for the sets C 1°, C 5°, and B 4°, and it will be seen that the dry weight of the juice exceeds the sum of the estimated constituents by 20–30 per cent.

The dry weight determinations may be as much as 2 per cent. too high, owing to the difficulty of complete desiccation *in vacuo* at ordinary temperatures. The salts present in the dried material amount to about 2 per cent. The sugars may be underestimated to the extent of 2–3 per cent. owing to loss during clearing, and to a further small extent owing to estimating as invert sugar a mixture of sugars in which fructose predominates. Even when these sources of error are allowed for, however, there still remains a discrepancy, which points to the existence of about 2–3 per cent. (of the total fresh weight of the pulp) of unidentified substance. A similar, but smaller, discrepancy has been found in the case of apples examined in this laboratory. Archbold (2) suggests that the substance in question is sorbitol, which has been reported as occurring in apples and pears, among other fruits, by Vincent and Delachanel (30) and Tutin (29).

V. Losses in the Constituents during Storage.

The quantities of the different substances disappearing during storage do not, of course, all represent material which is consumed in the respiratory processes of the fruit. The loss in total sugars and acidity is presumably due to respiration, but the reduction of the sucrose content by inversion does not represent a loss since the content of reducing sugars is thereby increased. No evidence is available to decide whether the break-down of the cell-wall provides material which is directly respired, or whether the break-down products merely accumulate. These break-down products are almost certainly of carbohydrate nature, derived from the pectic compounds. Pectin, according to the formula of Nanji, Paton and Ling (21) contains one arabinose group, one galactose group, and four galacturonic acid groupings, so that on break-down it could be expected to yield pentose and hexose molecules or break-down products of these. Attempts made during the present investigation to obtain evidence of any change in free pentose

content of the juice during the season were unsuccessful, the large hexose content vitiating the estimation by the Tollens method. If these reducing substances are not respired, their accumulation will increase the apparent concentration of sugars in the juice, and therefore reduce the estimated loss of sugar.

In order to study the relative amounts of some of the different constituents broken down during storage life of the fruit, curves of closest fit have been employed, as described by Haynes and Archbold (14). The rates of change of all the constituents, with the exception of the pectic compounds, have been represented by straight lines, i. e. the rates are constant. Small changes in rate may exist, but the error introduced into the calculation of the change in the amount of any constituent by regarding the rate of change as constant is negligible. The break-down of the pectic compounds is considered as following an exponential law in the case of sets B 4° and C 5°, as has already been discussed, but in set C 1° a straight line fits equally well. In the case of set C 1° the changes in the amounts of sucrose, fructose, and glucose practically ceased after 100 days in store, and they are, therefore, represented by two lines, as will be seen from Fig. 1 (*h*, *j*, *k*). The change in any constituent over a given period is determined by the difference between its value on the curve at the beginning and end of the period.

TABLE XIV.

Losses of Constituents (*per* 100 *grm. of tissue*) *in* 70 *Days in Store.*

Set.	Pectin + Proto-pectin.	Alcohol-insoluble Residue.	Total Sugar.	Sucrose.	Dry Weight of Juice.	Fructose.	Glucose.
B 4°	0·16	0·13	0·69	0·89	—	—	—
C 5°	0·30	0·27	0·31	1·20	0·41	+0·61	+0·27
C 1°	0·06	0·03	0·24	0·74	0·34	+0·19	+0·26

In Table XIV are given the changes in the amounts of some of the constituents, over a period of seventy days in store, the calculation being made from the point at which starch was no longer detected in the tissues, in sets C 5° and C 1°. In set B 4° the first estimation of residue was made after the fruit had been in store for twenty-nine days, and the calculation has been made from this point for all the constituents.

Comparison of Losses in Various Constituents.

(*a*) *Dry Weight and Sugars.* It will be seen that in sets C 5° and C 1° the loss of dry weight of the juice exceeds that of the total sugars. There is a net gain in the soluble pectin content of the juice during the season, and the loss of acid in these sets is negligible. The evidence,

however, is not sufficiently definite to decide whether there is any change in the amount of the unidentified substance already mentioned.

(*b*) *Sucrose, Fructose, and Glucose.* It has already been noted that there is an accumulation of fructose and glucose in each set, owing to the fact that sucrose is inverted more rapidly than the sugars are oxidized. In set C 5° the increase in the amount of fructose is exactly half the amount of sucrose inverted, i. e. the whole of the fructose produced by inversion accumulates, only glucose being used up in respiration. This is not the case in set C 1°, the increase in both fructose and glucose being less than the amount of each produced by the hydrolysis of sucrose, indicating that both have been oxidized to a certain extent. In this set rather more glucose than fructose accumulated. More information is, however, necessary before it can be decided with any certainty to what extent the temperature of storage affects the relative amounts of glucose and fructose consumed.

(*c*) *Alcohol-Insoluble Material and Pectic Compounds.* Since the alcohol-insoluble residue contains all the pectic substances present in the pulp, it is of interest to discover what relation the decrease in the total pectic content bears to the loss of residue. Unless the conversion of protopectin to soluble pectin is accompanied by the splitting off of some compound soluble in alcohol, this process should involve no loss of residue, since the soluble pectin itself is insoluble in alcohol. The further break-down of soluble pectin to sugars would, however, result in a decrease in the alcohol-insoluble residue. It will be seen from Table XV that in each set the decrease in the total pectic constituents is slightly larger than the loss of residue. It therefore seems fairly certain that apart from the initial loss of starch, the only components of the cell-wall to undergo break-down are the pectic compounds, and, furthermore, that no alcohol-soluble substance is split off in the production of soluble pectin from protopectin. If this were not the case, the loss of alcohol-insoluble material would be greater than the decrease in the pectic compounds.

The slight excess of the loss of pectic compounds over that of residue is probably not significant. On the other hand, since it occurs in each set it may indicate that the pectic compounds do not break down entirely to alcohol-soluble substances, but that a very small amount of some compound insoluble in alcohol is formed. In this connexion it is interesting to note that Candlin and Schryver (4) have prepared a substance having the properties and composition of a hemicellulose [1] by the action of weak alkalis on pectic acid. They have suggested that the hemicelluloses which occur in small quantities in plant tissues are break-down products of the pectic

[1] The 'hemicelluloses' are described (22) as water soluble, alcohol insoluble substances, giving a strong reduction with Fehling's solution after hydrolysis with HCl, and containing pentose and other furfural yielding groups. They differ from the pectic compounds in giving no soluble calcium compound, either before or after treatment with dilute NaOH.

compounds, and it seems possible that some substance of this nature may have been produced in the pears.

Keeping Properties and Losses in Constituents. It has already been noted that the length of the storage life was very different in the three sets of pears. Reference to Table XIV shows that the total consumption of respirable material, measured by the sum of the losses of total sugars and alcohol insoluble material (and excluding the very small loss of acidity and possible small loss of unidentified substance), is greatest in set B 4°, and least in set C 1°. It has been shown (14) that in apples of the same variety, grown on different soils, and stored at the same temperature, a rapid rate of loss of respirable material is, in general, correlated with poor keeping qualities. However, set B 4° with the largest loss, kept much better than set C 5°. It is probable that the relative losses of sugar and cell-wall material (i. e. pectic material) are very important in determining the length of storage life. In set C 5° there was more pectic break-down than in either of the other two sets, and it seems possible that the oxidation of the sugars in the juice was to some extent replaced by the break-down of pectic material, and that this replacement did not favour keeping. Whether the products of pectic decomposition are respired, or whether they merely augment the sugar concentration in the juice, the rate of break-down of the fruit in store appears to be definitely related to the rate of decomposition of the pectic substances, and the very marked retardation of ripening of pears in storage at 1° C. as compared with those of the same picking stored at 5° C., is probably chiefly due to the retardation of the pectic changes, since there was far less difference in the behaviour of the other constituents at the two temperatures. It seems probable that it is the marked effect of the pectic changes on the physical condition of the fruit which makes them such an important factor in the determination of the storage life. The changes in the condition of the cell-wall, which must accompany the pectic changes, probably affect the rate of gaseous exchange, and hence the whole metabolism of the fruit.

The control of these changes is, therefore, a most important condition for the satisfactory storage of pears, and an investigation of the behaviour of the pectic enzymes under different conditions would help in the elucidation of storage problems.

VI. Comparison of Sound and Unsound Pears.

(*a*) *Pears suffering from Softening and Over-Ripeness.* After four months in store at 4°, the remaining pears of set B showed a wide variation in condition. The fruit was, therefore, divided for analysis into two sets, in order to gain further information on the conditions which favour keeping. Set 1 consisted of fruit showing no signs of browning of the pulp, although

fully ripe, and set 2 consisted of fruit with brown, very soft pulp. Pears which showed evidence of fungal attack were not included in the samples. The results of these analyses are given in Table XV.

TABLE XV.

Comparison of Sound and Over-Ripe Pears—after 134 Days in 4° Store (1925-6).

	Sound. % of Original Fresh Weight.	Over-ripe. % of Original Fresh Weight.
Loss of Weight	18·7	22·9
Pectin + Protopectin = P	0·40	0·33
Soluble Pectin	0·19	0·16
Protopectin	0·21	0·17
Residue = R	2·28	2·19
Stone cells	0·42	0·41
(R − P)	1·88	1·86
Pectin in juice = Pj	0·21	0·14
Specific gravity of juice	1·055	1·057
Viscosity of juice	2·049	1·866
Dry weight of juice (Calculated) = Wj	10·67	10·46
Acidity = A	0·070	0·071
Total sugar = St	8·17	8·10
Reducing sugar	6·98	7·36
Sucrose	1·19	0·74
Wj = (St + A + Pj) (i.e. unestimated substance)	2·24	2·15

The analyses show quite definitely that the rotten pears had a smaller content than the sound ones of all the constituents, except acid and reducing sugars. The difference in total sugars was, however, very small. The bad pears showed a low content of sucrose, and a high reducing sugar content compared with that of the sound fruit. It is improbable, however, that this was due to a higher rate of inversion in the former, without a correspondingly high rate of oxidation, since no increase in the rate of inversion has been observed in other cases either in pears or apples (14) as the proportion of unsound fruit in the mixed sample increased.

The acidity in the two sets was equal.

It will be seen that the difference in pectic content entirely accounts for the residue (i.e. cell-wall) difference between the sound and unsound fruit. It is, of course, impossible to decide from these data whether the differences in composition are due to initial differences in the amounts of the constituents, or to differences in the rates of change, but it has already been noted that in apples a rapid rate of loss has been correlated with poor keeping properties. The bad pears had lost more water by transpiration than the sound ones, and it has already been mentioned that Luthra (18) found that a high rate of transpiration was associated with a rapid rate of break-down of the fruit. It has been suggested that the rapid transpiration, by keeping up the concentration of the constituents in the fruit, tends to

prevent the decline in the rate of chemical change which would otherwise occur as the amount of the substance undergoing change diminished.

(*b*) *Pears affected by 'Brown Heart'.* It is interesting to note here that although the normal browning and softening of the pulp are accompanied by marked changes in the pectic compounds, this is not a necessary accompaniment of all forms of break-down of the tissues. Table XVI gives the results of an analysis of a set of pears suffering from 'brown heart', a disease which occurs when the CO_2 content of the store is too high, and which is characterized by browning and drying of the pulp round the core (Kidd and West (16)). The affected tissue is firm, whereas in the case of over-ripe fruit it is very soft.

TABLE XVI.

Comparison of Sound and 'Brown Heart' Pears.

Condition of Sample.	Specific Gravity of Juice.	Pectin + Proto-pectin.	Soluble Pectin.	Proto-pectin.	Acidity.	Residue.
Sound	1·048	0·47	0·30	0·17	0·204	2·77
Brown-heart	1·047	0·48	0·23	0·25	0·155	2·57

The analysis showed no difference in total pectic content between sound pears and those attacked by brown heart, the development of soluble pectin from protopectin being, in fact, retarded in the diseased fruit. These observations were confirmed by microscopic examination of sections stained with ruthenium red.[1] No differences were detected in the appearance of the stained pectic substances in the two sets. The pears suffering from 'brown heart' exhibited a deficiency in acidity and residue. In the case of over-ripe pears, the deficiency in residue as compared with sound fruit was entirely accounted for by the smaller pectic content of the unsound pears, but in the set affected by 'brown heart' there was no such difference. Break-down of the non-pectic part of the cell-wall must, therefore, have occurred in the fruit affected by 'brown heart'.

The author has been informed by Dr. A. S. Horne that in the case of apples suffering from 'brown heart', which he has examined microscopically, no pectic disturbance has been detected.

It has already been shown that storage of apples in an atmosphere containing a large percentage of CO_2 (9 per cent.) retards the pectic changes. It is, therefore, not surprising that in a disease such as 'brown heart', caused by too high a percentage of CO_2 in the storage chamber, there should be no pectic break-down.

[1] The author is indebted to Dr. A. S. Horne for help with the microscopic examination.

VII. Comparison of Chemical Composition and Changes during Storage of Pears and Apples.

It is of interest to compare the analytical and storage results obtained in the case of the pears with those obtained with apples in this laboratory. In Table XVII are shown the results of analyses of several different varieties of pears, which have been made in the course of the present investigation. These analyses were, with the exception of the Kieffer pears, all made on pears which had not reached full ripeness. For purposes of comparison a column is included, showing the limits of variation in composition of apples of different varieties, analysed by Evans (12).

It will be seen that there are no very striking differences in composition between the apples and pears. The most marked difference is in the acidity, that of the pears being much lower than that of all the apples examined by Evans, except the Worcester Pearmains. The sucrose content of pears is low compared with that of most of the apples. The amount of alcohol insoluble material is, on the whole, higher in the pears, but this is very largely due to the presence of stone-cells rather than to much thicker cell-walls.

Behaviour during Storage. In general, the changes in the chemical constituents occurring during ripening and breakdown in store are similar in pears and apples. In both, the sugars provide respirable material; in the case of most apples the acid provides further material for respiration (14), but in the Conference pears very little acid was lost in this way.

Pears lose more weight by transpiration during storage than apples which have been examined in this laboratory, and this difference is especially marked in 1° storage. A set of Bramley's Seedling apples studied during the season 1926–7 lost 1·4 per cent. of their weight during the first fifty days in 1° store, while the Conference pears stored at 1° at the same humidity lost 13 per cent. in the same time. This large loss of weight has already been discussed, and is probably chiefly due to a larger number of lenticels in the pear. In addition, differences in the external coating of wax on the fruit probably contribute to the transpiration differences.

It has been shown that in the pear the residue, i. e. the cell-wall, losses during storage are entirely accounted for by the break-down of the pectic compounds, the non-pectic part of the cell-wall being unattacked. Some preliminary results obtained in a former season suggested that this was not the case for acid apples, such as Bramley's Seedling, and this idea has been confirmed by the results obtained from weekly estimations of the pectic content and alcohol-insoluble residue[1] carried out on Bramley's Seedlings

[1] The results for alcohol-insoluble residue have been kindly furnished by Miss H. K. Archbold.

TABLE XVII.

Comparison of Chemical Composition of Pears and Apples of Different Varieties.

Variety.	Protopectin + pectin.	Residue.	Total Sugar.	Sucrose.	Acidity.	Stone Cells.	Fructose. / Glucose.
Conference (three seasons) .	0·51	—	—	—	0·086	—	—
	0·63	2·36	9·09	2·32	0·092	0·40	—
	0·62	2·50	8·16	2·25	0·071	—	3·3
Vicar of Winkfield . . .	0·65	2·60	10·12	0·86	0·242	0·32	—
Catillac	0·73	2·95	9·11	0·72	0·216	0·48	—
S. African pears (var. unknown)	—	2·15	8·45	—	0·103	—	—
Kieffer hybrid	0·47	2·77	—	—	0·204	—	—
Limits of variation in apples (reprinted from Evans (12) .	0·76 (Cox's Orange Pippin)	2·55 (Royal Russet)	12·67 (Worcester Pearmain)	5·55 (Russet)	1·27 (Bramley's Seedling)	—	5·77 (Grenadiers)
	to	to	to	to	to	—	to
	0·44 (Lane's Prince Albert)	1·71 (Emneth)	8·35 (Grenadiers)	1·78 (Annie Elizabeth)	0·191 (Worcester Pearmains)		2·56 (Annie Elizabeth)

stored at 1° C. The total percentage loss of pectic substances during the last 160 days in store (i. e. from the point at which the total pectic content began to decrease) was 0·05 grm., and the loss of alcohol insoluble material over the same period was 0·13 grm. The difference between these losses is large enough to be significant, and the loss in residue was not due to the disappearance of starch, since this was complete much earlier than the point from which the calculation was made. It is, therefore, possible that some degree of break-down of the cellulose constituents of the cell-wall took place. No further evidence is available to decide whether this difference in the break-down of the cell-wall is due to a difference in constitution

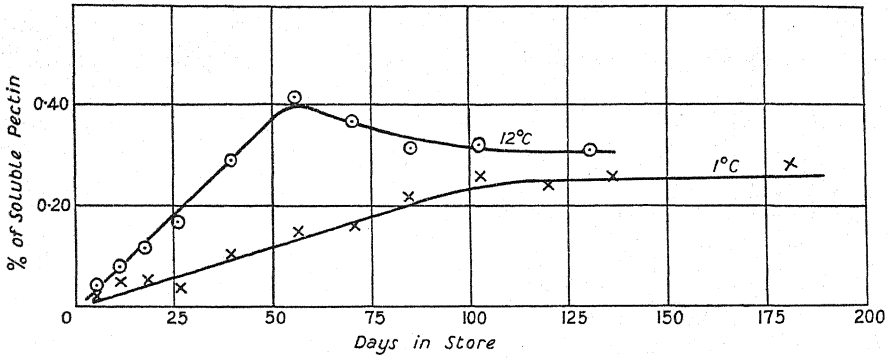


FIG. 5. Development of soluble pectin in Worcester Pearmains stored at 12° C. and 1° C.

between the cellulose compounds in the two fruits, or whether the high acidity in the apple in some way renders the cellulose constituents more readily attacked.

Differences in Pectic Changes. In both apples and pears the softening which takes place during ripening and storage is associated with the hydrolysis of protopectin and consequent development of soluble pectin, but it is to be noted that there is a very marked difference between the rates at which the pectic changes take place, and the effect on them of storage at different temperatures. It has been shown that Conference pears stored at 12° C. exhibit a very rapid development of soluble pectin, the maximum being reached in about a fortnight, and complete break-down and decay of the fruit taking place in three weeks. Carré (5) has shown that Lane's Prince Albert apples stored at 12° C. exhibited their maximum development of soluble pectin after two months, and remained in good condition for another month, after which slow break-down set in, and the analyses were discontinued after seven months.

It is surprising to find that storage at 1° C. retards the production of soluble pectin in pears far more than in apples. It has already been shown that there is very little softening of Conference pears in cold store, and only a small increase in the soluble pectin content during six months storage.

Cox's Orange Pippins and Lane's Prince Albert apples, studied by Carré, showed a retardation of from six weeks to two months in the production of the maximum amount of soluble pectin in 1° store, as compared with fruit stored at 12°, and at both 1° and 12° the maximum content of soluble pectin attained was the same. Considerable development of soluble pectin was also found to take place in Bramley's Seedling apples in cold store, and this has been confirmed by the present writer. On the other hand, in a set of Worcester Pearmains examined during the present season (1927–8) the maximum soluble pectin content (0·4 per cent.) was attained after about eight weeks at 12° C., and although there was some production of this substance in the fruit stored at 1°, the soluble pectin content had only reached 0·28 per cent. in thirty-one weeks. The results of these analyses are given in Table XVIII, and shown graphically in Fig. 5.

TABLE XVIII.

Development of Soluble Pectin in Worcester Pearmains stored at 12° and 1° C.

Days in Store.	Soluble Pectin Content. 12° Store.	1° Store.
0	0·02	0·02
8	0·06	0·03
14	0·08	0·04
21	0·14	0·03
28	0·19	0·03
50	0·40	0·15
63	0·42	0·14
77	0·31	0·17
91	0·32	0·26
113	0·33	0·24
126	—	0·26
147	0·30	0·27
217	—	0·28

The protopectin in the pear is, therefore, more readily broken down at the higher storage temperatures, and much less readily attacked in 1° C. storage than that in the apples examined. The further break-down of pectin into alcohol-soluble substances, when the fruit becomes over-ripe, takes place more rapidly at 12° C. in pears than in apples, and in cold storage it is exceedingly slow in both cases.

No entirely satisfactory explanation of these differences can be attempted without further investigations of the mechanism of the pectic changes. Carré and Horne (8) made periodical microscopic examinations of sections of the Conference pears stored at 4°, and found that in general no striking differences could be detected in the behaviour of the pectic material during ripening and break-down between pears and apples. The chief difference

was one of rate, the pectic changes occurring much more rapidly in the pears. They assume that the pectic changes are brought about by enzyme activity, and suggest that acidity is the controlling factor, and that a low acid content 'involves a lessening of the protoplasmic control of the enzymes producing pectic decomposition'. They quote in support of this theory the fact that certain varieties of apples with a low acidity are those which most rapidly exhibit the tissue softening which accompanies pectic break-down. It may be noted that in the experience of the present writer this statement is only true when the apples are stored at ordinary temperatures. In cold storage tissue softening and pectic break-down are more retarded as compared with fruit stored at 12° C., in apples of low acidity than in acid apples, as is shown by the results already given for Worcester Pearmains, and this occurs to a still greater extent in the pears.

It is probable that the chemical combination in which the protopectin exists in the cell-wall differs in the two fruits. Even if this were the case, it would be expected that if the compound in the pear were more readily broken down than that in the apple at the higher storage temperatures, a similar relationship would obtain in cold storage.

It seems possible that two agents may be concerned in the conversion of protopection to pectin—an enzyme and the acid present. The conversion is readily effected by dilute acids, and it is, therefore, quite possible that the acid present in apples such as Bramley's Seedling (1·27 per cent.), Lane's Prince Albert (0·92 per cent.), and Cox's Orange Pippin (0·66 per cent.) may play the chief part in the process during ripening. It is not suggested that there is a complete absence in these apples of an enzyme capable of converting protopectin to pectin. Carré has, in fact, made some preliminary experiments on the development of soluble pectin in washed-out apple tissue kept at laboratory temperatures, the results of which indicate the presence of such an enzyme (5). Its action is, however, probably inhibited by the acidity, at any rate at the beginning of the storage season. In the pears and in such apples as Worcester Pearmains, having a very low acidity (0·30–0·19 per cent.), the enzyme is probably the more important factor in the development of soluble pectin. The effect of temperature would in this case undoubtedly be greater than on the acid-controlled similar process in acid apples, and this would account for the greater differences in the rate of pectic change at 1° and 12° C. observed in fruit of low acidity.

In support of this suggestion, the work of Overholser and Taylor (24) already referred to may be noted. They found that in the case of pears there was an optimum temperature (70–85° F.) for the rate of ripening in store, above which point there was a distinct retardation of ripening. Such an optimum point was not found in the case of the apples examined by them (Yellow Newtowns). This would be expected if the ripening pro-

cesses in the pear were chiefly enzymic, while those in the apple were chiefly determined by the acidity.

If this explanation is correct, some further factor, possibly some change in the permeability of the plasma membrane, must account for the fact that in very immature apples, where the acidity is at its maximum, there is no development of soluble pectin.

VIII. Summary.

Analyses have been made of pears (variety Conference) stored at 12°, 5°, 4°, and 1° C. The methods used for the estimation of the various constituents of the pears are described. In the estimation of soluble pectin, the necessity for purification of the extracts by centrifugalization before precipitation as calcium pectate has been shown. Small quantities of a substance precipitable in the same way as pectin have been extracted with NaOH from the tissues, after the extraction of pectin and protopectin with HCl. This substance probably corresponds to that similarly obtained from apples by Carré, and described as a pectic substance from the middle lamella. This method of extraction has been shown to be unsatisfactory as a quantitative method.

Determinations have been made of the sampling error involved in estimating the different constituents.

Marked differences in the rate of ripening of the fruit at different temperatures were observed. Whereas at 12° C. ripeness was attained in ten to twelve days, at 1° C. the fruit showed scarcely any signs of ripening after six months. The connexion between the marked wilting observed in immature pears stored at 1° C. and the effect of low temperature on the lenticels and skin is discussed.

The differences in rate of ripening at different temperatures were accompanied by similar differences in the rates at which the pectic changes took place, rapid ripening being associated with rapid decomposition of the pectic substances. The rate of conversion of protopectin to soluble pectin during ripening, and the further break-down of soluble pectin, followed the exponential law in the case of the fruit stored at 4° and 5° C., but at 1° C. the pectic changes were exceedingly slow, very little soluble pectin being developed during the whole storage period, and the rate of change appeared to be practically constant.

During the ripening period changes in the viscosity of the juice are shown to be due to changes in the content of soluble pectin. After the period of full ripeness is past, there is a more marked decrease in viscosity than in pectic content. This may be due either to the disappearance of some other colloid present in the earlier stages, or more probably to some physical or chemical change in the soluble pectin. It is suggested that this change is that of demethoxylation of the neutral pectin.

It is shown that the development of soluble pectin is the chief factor concerned in the softening of the fruit. Both the dry weight of the juice and the total sugars decreased at a practically constant rate during the whole storage life. The rate of inversion of sucrose was also practically constant, except at 1° C. at which temperature inversion ceased after three months. There was an accumulation of reducing sugars in each set, owing to the rate of inversion being greater than the rate of oxidation of the sugars.

The acidity of these pears was very low and individual variations large, but there is some evidence of a slight decrease in the acid content during storage.

There is evidence of the presence in the juice of a considerable amount of some unidentified substance of non-reducing and non-acidic character. It is suggested that the substance in question may be sorbitol.

The losses in some of the constituents during storage have been found from curves of closest fit calculated to the experimental points. In the fruit stored at 5° C. the whole of the fructose produced by inversion accumulated, only glucose being consumed. At 1°, however, both glucose and fructose were consumed.

The break-down of the pectic compounds is shown to account for the whole of the decrease in the alcohol-insoluble material. No evidence is available to decide whether the products of pectic break-down accumulate or are consumed.

It is shown that in pears differences in keeping quality are in all probability due mainly to differences in the rate of break-down of the pectic compounds.

Comparison of sound pears with those of the same picking which broke down, has shown that the latter had the smaller content of all the constituents except reducing sugars and acid. It is suggested that the more rapid transpiration rate in the fruit which broke down favoured the more rapid chemical change of the constituents by keeping up their concentration. The pectic content of pears suffering from brown-heart was found to be same as that of sound fruit of the same set.

Pears and apples are shown to be similar in most respects in chemical composition, and the changes in the constituents occurring during ripening and break-down. The chief difference lies in the changes in the cell-wall constituents, there being some evidence that in apples, substances other than those of pectic nature are broken down.

The pectic changes take place much more rapidly at ordinary temperatures (12° C.) and more slowly at 1° in the pears than in any apples which have been examined. The possibility that this may be due to a difference in the agents concerned in the conversion of protopectin to pectin in the two fruits is discussed, and it is suggested that in the case of apples of high

and moderate acidity this agent is the acid, while in the pear and in apples of low acidity the process is chiefly enzymic.

The author wishes to thank Professor V. H. Blackman and Dr. D. Haynes for their valuable criticism and advice.

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Notes on the Chondriosome-like Bodies in the Cytoplasm of Equisetum.

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With Plates V and VI.

IN a series of Studies on the structure of plant protoplasm, of which two have now been completed (Bowen 2, 3), I am attempting the collection of data upon the basis of which conclusions may perhaps ultimately be drawn, concerning the homologies presumably existing between the structural elements of the plant and animal cell. In the Studies thus far completed two general kinds of cytoplasmic elements have been considered. The first of these are of a very special nature, in that they are not demonstrable by ordinary methods of technique. If one wishes to study these bodies, which I have temporarily called the osmiophilic platelets, it is necessary to have recourse to the methods of osmic impregnation so familiar to workers on the animal Golgi apparatus. The other kind of cytoplasmic elements differ markedly from the osmiophilic platelets in the fact that they are more or less completely demonstrated by many of the more common cytological methods. These bodies are variously shaped, but in a general way they fall within the forms assumed by the chondriosomes in animal cells; and furthermore, in meristem cells at least, they can be well preserved and stained only by the methods primarily devised for the demonstration of the animal chondriome. Hence, to distinguish this general group of elements, we may call them chondriosome-like bodies, with, however, the specific reservation that this name is meant to indicate a superficial resemblance only. As to whether these bodies represent a single category of formed materials or are of a heterogeneous nature, nothing is implied in the name. Nor is the name intended to imply that all or any of them are necessarily equivalent to the chondriosomes of animal cells.

An extended study of all the plant groups except Thallophyta, which have not yet been examined, has revealed in the plant cell the constant occurrence of osmiophilic platelets. The morphology and general behaviour

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of these bodies has been fully described in previous papers (Bowen 1, 2, 3), and need not be considered here. I want merely to point out that these platelets have been very clearly demonstrated in the root-tips, and in the growing points of a pteridophyte, *Equisetum* (Bowen 2).

In my second Study on the plant cell (Bowen 3), the chondriosome-like bodies of the angiosperms have been extensively examined. As a result of this investigation, I have reached the conclusion that, in the cells of angiosperms as represented particularly by root-tip meristem, the chondriosome-like bodies are primarily divisible into two distinct and quite separate categories. These are separable on the basis of differences in staining behaviour and in morphology and structure. One class is definitely associated with the production of plastids of all kinds, the other class is clearly not connected in any way with this operation. The bodies in a single cell which can produce plastids, I have called collectively the plastidome. In meristem cells, where no plastids are recognizable, the elements of the plastidome are termed proplastids or archiplasts, because they represent the primordia from which all types of plastids can be differentiated. In the species which I have studied, the proplastids are characterized by a shape which is predominantly elongate (rod- or thread-like). This is sometimes replaced by a more compact or rounded shape, but it is the elongate type which is really typical. The other class of chondriosome-like bodies, the class not interested in plastid formation, has been temporarily called the pseudochondriome. This name is intended merely to indicate that these bodies look and behave much like animal chondriosomes. They are characterized in meristem cells by a rather accurately spherical shape. They are in general markedly smaller than the proplastids. Their shape is also distinctly different, and elongate forms are rarely found in angiosperm meristem. Sometimes bacillus-like shapes are assumed and various dumb-bell forms, apparently connected with a division process. Chains of three, rarely more, granules may occur, but these are not abundant. The function of these elements is entirely unknown.

The chondriosome-like bodies of angiosperm cells are thus more or less easily divisible into two definite and distinct categories—the plastidome and the pseudochondriome. This conclusion is in harmony with the essential findings of many plant cytologists, for example, Rudolph (19), Scherrer (21), Sapĕhin (20), Mottier (16), Guilliermond (9), Emberger (6), Mangenot (12), Dangeard (5), and others. In one respect, however, the views held by these observers are not in harmony—they differ as to the interpretation of the two categories of bodies in terms of the animal chondriosomes. Most of the authors mentioned agree that some of the chondriosome-like bodies in plant cells actually are homologous with animal chondriosomes. The elements thus agreed upon are the granular class which I have called the pseudochondriome. Guilliermond, Emberger and Mangenot, on the other

hand, hold that the plastidome is also mitochondrial in nature. In their opinion the chondriosome-like bodies of the plant cell are actually all chondriosomes, but are functionally divisible into two classes which are always distinct from each other. My own studies have revealed no adequate basis upon which the name chondriosome may be definitely and finally applied to any class of bodies in the plant cell. The evidence, not yet of a critical nature, suggests that the pseudochondriome really is the homologue of the animal chondriome, as several botanical workers have held, but I have left this question open for possible decision later on.

Material and Methods.

It is the purpose of this paper to extend my observations on the chondriosome-like bodies to pteridophytes. For this purpose I selected *Equisetum* primarily because of the large apical-cells which it is known to possess. Masses of *E. arvense* which had been kept in pots in the Columbia green-house, were separated and the roots re-bedded in fresh earth. An abundant new growth was obtained in about two weeks. Both root-tips and the young growing-points were preserved for study. In the case of root-tips, they were simply rinsed in tap-water, and then cut off and placed at once in the fixative. The young growing-points were cut off and placed quickly in the fixative. Very young ones are the best, not only because of their small size, but also because they sink more readily and afford better fixation. However, in no case did I get good fixation of the meristematic tip of the main stem. Young lateral buds, which present the same general features, gave much better technical results.

The methods used are those already noted in my previous papers. Since the sorting out of the plastidome and pseudochondriome depends in many cases upon the technical method employed, I have appended to the description of each figure in the Plates, an exact statement of the method by which the original preparation was obtained. The formula used in the case of osmic-impregnation methods is explained in my first Study (Bowen 2), and additional notes are given in the succeeding Study (3). It does not seem worth while to repeat these explanatory details again, since the originals are readily obtainable.

The methods used fall readily into two groups:

(1) Osmic impregnation methods devised by zoologists especially for the demonstration of the animal Golgi apparatus.

(2) Methods devised especially by zoologists for the demonstration of the animal chondriome.

The osmic methods employed were those of Kolatchev and of Weigl. They were carried out exactly as described in Study I, indeed the same preparations were used for the study both of platelets and chondriosome-like

bodies. These methods gave no results on either the pseudochondriome or plastidome in *Equisetum* root-tips. In growing-points no results were obtained on the pseudochondriome, but the plastidome was occasionally blackened, especially by the Weigl method. In such cases there is often a simultaneous blackening of the osmiophilic platelets. The best results on the plastidome were on plastids proper, the osmic methods being of little use on meristematic cells. In general, osmic impregnation proved of little real value in the analysis of the chondriosome-like bodies in *Equisetum*. It never produced preparations in any way comparable with the striking results often achieved on angiosperm material (see Study II).

Of the mitochondrial variations, I tried three particular stains, the Benda crystal violet method, the fuchsin-thionin-aurantia of Kull, and the orthodox Fe-haematoxylin. These stains were combined with various fixatives, more particularly Mottier's (16) modification of Benda's Flemming, Champy, and occasionally Meves' fluid for plants. For Fe-haematoxylin staining, the material was not mordanted after fixation, but for the Benda and Kull stains, the material was usually postchromed according to the methods commonly given in connexion with these stains. The Benda method proved rather unusually successful, especially on root-tips. It was combined (after proper postchroming) with all three of the fixatives mentioned, the best results being obtained with Mottier's fluid (fixation for about forty-six hours). With the Kull stain, the fixation was almost always in Champy (for twenty-four hours), with postchroming as given by Kull for this method.

The Benda stain proved particularly useful for the selective staining of the plastidome at all stages and in all kinds of cells. The pseudochondriome was very rarely stained by this method. This corresponds with the result which I obtained very generally in angiosperm root-tips. On the other hand, the Kull method was essential for the differentiation of the pseudochondriome. This result is again in correspondence with my earlier results on angiosperm root-tips, but in the case of *Equisetum* the Kull stain was often unsuccessful, and in many cases also stained the plastidome either wholly or in part. Occasionally it gave a very good stain of the plastidome with no result whatever on the pseudochondriosomes. Fe-haematoxylin often stains both kinds of bodies, but in general is much less successful than either of the more specialized techniques.

Observations.

As is evident from the preceding sections, I have already anticipated the conclusions of this paper to the extent of indicating that in *Equisetum*, as in the angiosperms, the chondriosome-like bodies are divisible into two categories, the plastidome and pseudochondriome. This subdivision much

simplifies the description of my findings, and so seems to justify the making of the distinction prior to the exposition of the grounds upon which the separation is based.

The Root-tip.[1]

The Benda method permits the absolute separation of the plastidome from all other cytoplasmic structures at practically all stages in the root-tip proper. The only exception is in the older cells of the root-cap where, as already noted in angiosperms, the pseudochondriosomes are sometimes demonstrated by this and other methods. In these cells, however, the plastidome is already in the form of well-developed leucoplasts, and no possible confusion can arise as to the identity of the two kinds of elements.

The most difficult place in the early root-tip to obtain a good stain is in the apical-cell. Here, for some reason, the crystal violet always takes less intensely, although in the adjacent cells the stain may be very sharp. In Pl. V, Fig. 7 is shown an apical-cell. There is a large nucleus with several heavily stained nucleoli. The plastidome occurs in the form of small archiplasts, which tend to lie in proximity to the nucleus. Their shape is varied, but an irregularly rod-like form is frequent. Granular shapes are not characteristic, and where such seem to occur, it is usually due to the fact that rod-shaped proplastids are being viewed more or less on end.

Passing from the apical-cell into the root-cap area,[2] a cell very close to the apical-cell is shown in Fig. 1. The archiplasts are here very distinctly elongated bodies, sometimes almost thread-like. Every stage in the gradual transformation of these bodies into the large leucoplasts so characteristic of the older root-cap cells can be followed with diagrammatic clearness. Various steps in the earlier phase of leucoplast development are shown in Figs. 2 to 4; and in Fig. 6 some individual leucoplasts at a higher magnification. The general course of events is identical with the conditions which I have described in *Vicia* (Bowen 3).

Passing now from the apical-cell in the opposite direction, very clear pictures of the plastidome are everywhere obtainable. Fig. 8 is from a large cell just a little above the apical-cell. The archiplasts are characteristically elongate, but they are very delicate and much more decidedly polymorphic than is the case in angiosperms. Figs. 9 and 10 are cells from the early plerome border. The proplastids are less clearly elongate and even more varied in shape. In Fig. 11, from a slightly higher level, a tendency towards a more elongate shape is noticeable. In Fig. 12, from the central plerome

[1] On page 717 of my first Study (Bowen, 2), the statement is made that the *Equisetum* root-tip contains a central core of large cells somewhat like those of *Hordeum*. Since this statement may easily be misunderstood, it should be tempered to the extent of saying that, while there is a central core of cells in *Equisetum*, it is of a much less conspicuous nature than in *Hordeum*.

[2] The general anatomical subdivisions of the root-tip will be followed as given in Study II (Bowen, 3).

the same general type of archiplasts occurs. A similar cell at a somewhat higher level is shown in Pl. V, Fig. 13, and here the tendency toward the development of elongate archiplasts is very clearly evident. The same thing is shown in Fig. 14, a telophase stage in the division of a cell like that of Fig. 13. Older stages in the plerome cells I have not studied, but it is to be noted that no evidences of leucoplast development have yet appeared. Whether such a transformation occurs at a higher level in the root has not been determined, but it may be noted in passing that the development of leucoplasts in the plerome of angiosperm root-tips regularly occurs at a level above that at which leucoplasts appear in the periblem. The same thing is probably true in the pteridophyte root.

Returning again to the apical-cell, the course of events in the dermatogen can be similarly traced. In Fig. 15 is a cell from the dermatogen very close to the apical-cell. In general appearance the archiplasts are similar to those of the plerome cells. Here, however, the transformation of the archiplasts into leucoplasts begins very early. This is marked by a change in the morphology of the proplastids, resulting in a tangle of somewhat swollen forms. Fig. 16 shows the very beginning of leucoplast development, and Fig. 17 a more advanced stage. Both figures are taken from tangential sections which do not include the nucleus, and by their thinness somewhat simplify the resulting pictures. The leucoplasts in these cells, when completed, have a characteristic shape which is quite typical, and quite unlike any I have seen in angiosperms. When viewed at the most favourable angle they have a fusiform shape—already noted by Emberger (6).

Again returning to the apical-cell, the course of events in the periblem is essentially the same as in the dermatogen. Fig. 18 shows a cell from the outer layer of periblem just before the appearance of starch in the proplastids. The morphology of the plastidome almost defies description, but the Figure shows the general features in a most typical way. In Fig. 19 leucoplasts have been formed, and in Fig. 20 are shown examples of leucoplasts from the older periblem (at a higher magnification). They do not differ particularly from those in angiosperm cells.

The history of the plastidome is thus essentially identical with the events which I have fully described in the angiosperm root-tip (Bowen 3). By the proper application of the Benda stain, it has been possible to separate the plastidome with the greatest sharpness, and to follow practically every step in its history. It is again notable that in plastid formation the entire plastidome is involved, and no unchanged archiplasts remain in cells with differentiated leucoplasts. This is only what might be expected from the conditions in angiosperms, where a similar 'all or none' transformation of the plastidome has been described by most workers.

The pseudochondriome is much more difficult to stain selectively, and

while the Champy-Kull method was very successful at certain levels, in other parts of the root-tip, clear-cut results were not obtained. Thus in the apical-cell and in the early portions of plerome, periblem and dermatogen, I could not stain the pseudochondriosomes by themselves. When the plastidome also is coloured the preparations are so complicated as to be useless for the kind of analysis in which I am interested. In older cells of all kinds, however, the identity of the pseudochondriosomes is very clear, and their morphology and general behaviour is exactly like that already described in angiosperm root-tips. In the older root-cap cells, for example, the pseudochondriosomes are sharply separated from the leucoplasts, which may themselves be practically invisible (Pl. V, Fig. 21). The pseudochondriosomes have usually a spherical shape, sometimes slightly elongated, and frequently showing stages in apparent division. They are scattered throughout the cytoplasmic area. With the Benda stain also, the pseudochondriosomes can sometimes be stained in the older cells of the root-cap (Fig. 5), and in such cases they usually appear smaller than when demonstrated by the Champy-Kull technique. A similar behaviour towards various technical procedures has been noted in angiosperm root-tips.

In the plerome (Fig. 22) and periblem (Fig. 23), the appearances in Champy-Kull preparations are identical with those in the root-cap. One might, indeed, interchange cells from any region in the root-tip for any other part of the root without in any way disturbing the validity of the picture as regards the individual pseudochondriosomes. Or, on the other hand, one might substitute a cell from any region of an angiosperm root-tip without introducing any note of discord in the *Equisetum* picture. In brief, the pseudochondriosomes in the root-tips of angiosperms and pteridophytes (so far as studied) are practically identical in shape and distribution, and in all cases they present the same absence of any visible evidences of functional activity. It is impossible to doubt that we are dealing with the same type of elements in all these cases. As regards the detailed structure of the pseudochondriosomes, in my preparations I only rarely saw the differentiation into outer and inner portions so clearly seen in angiosperms (Bowen 3). With further staining trials, I believe that results similar to those in the angiosperm root-tip would probably be obtained.

The distinction between plastidome and pseudochondriome is brought out very clearly in cells where leucoplasts are differentiated. In Fig. 23, for example, a group of faintly stained leucoplasts in the lower left-hand corner of the cell stands in obvious contrast to the surrounding pseudochondriosomes. Fig. 24 is a minute cytoplasmic fragment drawn at a higher magnification showing one plastid and several neighbouring pseudochondriosomes. The morphological and functional distinctions are obvious.

I have not particularly studied the phenomenon of mitosis. The archiplasts show some relation to the spindle figure but one by no means so

striking as in angiosperms. This is probably correlated with the small size, irregular shape, and large number of the proplastids. Nevertheless, Pl. V, Fig. 14 of a telophase shows clearly that phenomena of orientation do occur in some degree, as indicated by the grouping of the proplastids in the daughter cells.

Summary.

In the root-tip of *Equisetum* there are two kinds of chondriosome-like bodies which, so far as can be determined, are absolutely distinct one from the other. One of these, the plastidome, has been followed in detail in all parts of the root-tip and it is found to behave exactly like the bodies of similar function in the root-tips of angiosperms. The individual archiplasts differ from those in angiosperms in their smaller size, much larger number, and particularly their more irregular and indefinite shapes. Elongate forms are, however, characteristic, and, as in the angiosperms, spherical forms are unusual. Accompanying the plastidome is another class of cytoplasmic components, the pseudochondriosomes. These are typically spherical in shape and do not undergo any significant changes in morphology in any part of the young root-tip. They have not been identified in all parts of the root-tip because of technical difficulties. But that they have no relation at any point with the archiplasts is indicated by the history of the latter bodies, which show nowhere any possible transition stages into pseudochondriosomes. The evidence, though incomplete in certain respects, practically demonstrates that the chondriosome-like bodies in the pteridophyte root-tip are essentially identical with those in the angiosperm root-tip as previously described (Bowen, 3). So far as morphology and function can be studied, the bodies identified as plastidome in pteridophyte and angiosperm, are homologous; and a similar conclusion follows in the case of the pseudochondriome.

The Growing-point.

Longitudinal sections of the very young growing-points of *Equisetum* permit a study of the apical meristem of the main stem or its lateral branches, of the main stem itself and the leaves, including vascular bundles, guard cells, &c. Cells of most of these types have been examined, and fairly complete results were obtained except in the case of the apical region of the primary stem. This is a matter of little concern, since the similar lateral branches offer favourable regions for study.

In the leaves all stages in the evolution of the plastidome can easily be followed. The Benda stain is best for the demonstration of the archiplast stages, but when plastids have begun to differentiate Fe-haematoxylin or acid fuchsin also yield satisfactory preparations. In certain parts of the outer layer of cells at the very base of a leaf, the plastidome is in the form

of proplastids (Pl. V, Fig. 25). These bodies are quite small and of rather indefinite shape. They tend to be somewhat drawn out and are practically never spherical. Thus in their general morphology they somewhat resemble the plastidome in the young cells of the root-tip. As one passes upward along the leaf, the archiplasts quickly become larger and more definite (Fig. 26), and soon exhibit obvious evidences of the onset of plastid development (Fig. 27). At a still higher level, young plastids appear in characteristic form (Fig. 28) and these are soon developed into the typical plastid of the more proximal part of the leaf (Fig. 29). Plastids of this type are often blackened by osmic impregnation. They are also very beautifully demonstrated by Fe-haematoxylin, as shown in Figs. 30–32. A leaf-cell with somewhat more developed plastids, is shown in Pl. VI, Fig. 50. In this case the plastid substance proper has been blackened by osmic acid in a most typical manner. Even more conspicuous plastids appear in Figs. 51 and 52 from the central area around a leaf base. In Fig. 51 the Champy-Kull method has demonstrated both plastids and pseudochondriosomes; while in Fig. 52 the Kolatchev method has demonstrated plastidome and osmiophilic platelets. A similar result with osmic impregnation is shown in Fig. 45, a portion of an old leaf-cell. In the differentiated cells located in the distal part of the leaf the plastids are very large (Figs. 46 and 48), and are often finely demonstrated by osmic impregnation, as well as by any of the usual staining methods.

The pseudochondriosomes in the meristematic type of cell shown in Pl. V, Figs. 25 and 26, is difficult to study for two reasons. In the first place, the plastidome is very apt to stain and the resulting picture is too complex for satisfactory analysis. In the second place, in the absence of demonstrated archiplasts it is difficult to determine the exact stage in which a particular cell may be. In any event, the staining of these undifferentiated cells is difficult, just as was found to be the case in the root-tip. However, fairly demonstrative examples of undifferentiated leaf-cells are shown in Pl. VI, Figs. 36 to 38. In Fig. 36 only the pseudochondriosomes are shown, but it is certain from the environment of the cell that the plastidome is still in the archiplast condition. In Fig. 37 a few archiplasts are faintly stained, and in Fig. 38 their differentiation is clear. A leaf-cell with plastids in an early phase of development is shown in Fig. 39. Fig. 40 corresponds roughly to Pl. V, Fig. 29, and Pl. VI, Figs. 41 and 42 are progressively older stages in the differentiation of the same type of cell. In all these examples the pseudochondriosomes are visible in exactly the same form and with the same distribution as in the root-tip. They possess the same typically spherical shape, with the same variations probably to be associated with a process of multiplication by simple division. As the plastidome alters progressively, the pseudochondriosomes remain unchanged. As soon as plastids are clearly differentiated, they usually also stain with acid fuchsin, and the two

components can be followed side by side through the differentiation of the leaf-cell, the special morphology of the plastids always making the distinction between them diagrammatically clear. Fe-haematoxylin preparations also often demonstrate the pseudochondriosomes together with the plastids (Pl. V, Fig. 31).

In older and larger leaf-cells, the pseudochondriosomes seem to be increased slightly in size (Pl. VI, Figs. 44 and 46). A characteristic feature of at least some of the older leaf-cells is the appearance of a normal polymorphism in the pseudochondriome. Instead of a constantly spherical shape, the pseudochondriosomes now exhibit more elongate types, sometimes distinctly rod- or even thread-like (Figs. 47 and 48).[1] This tendency to assume an elongate form has been observed by many other workers, and attention has been particularly directed to it in angiosperms by Guilliermond (9, for example). This change is in no way associated with the development of new plastids. All of the true archiplasts have been transformed long-since into plastids, and no reserve of archiplasts is ever left for later differentiation, and the elongate pseudochondriosomes never become plastid-forming bodies. The fact that in differentiated cells the pseudochondriosomes assume shapes reminiscent of the archiplasts is thus a mere parallelism, and is in no way indicative of any real relationship. Such obvious examples of the impossibility of drawing conclusions as to the nature of a body on the basis of mere morphology, have been consistently overlooked by those who have insisted that the chondriosomal origin of plastids is a demonstrable fact. The fact that elongate pseudochondriosomes look like archiplasts does not mean that they are archiplasts, as one can easily prove by direct observation; and by the same token, the fact that archiplasts look like animal chondriosomes does not at all prove that they are chondriosomes.

In the leaves there are two specialized classes of cells, those which make up the vascular bundles, and the guard cells. The guard cells are very large, and with Champy-Kull good examples were obtained of the pseudochondriome, the plastidome being left entirely unstained. Such a cell is shown in Fig. 49. The pseudochondriosomes are in every way typical. The cells along the vascular bundles show a very interesting condition of the plastidome. The archiplasts are in the form of very delicate threads which run for the most part parallel to the long axis of the cell (Fig. 34). As one passes out along the vascular bundle, these cells show the gradual transformation of the archiplast threads into plastids. These

[1] These figures from leaf-cells of *Equisetum* should be compared with Maximow's (15) Fig. 3, for example, from a living cell of the pumpkin. The similarity in morphology of the pseudochondriome in these widely separated plants is extraordinary. Of even more interest is the fact that one figure is drawn from a fixed and stained cell, the other from a living cell—yet the pictures are essentially identical.

phenomena in Pteridophyta, were first observed and fully described by Pensa (17, 18) in *Scolopendrium* and *Aspidium*, and my results are in essential agreement with his. Cells somewhat further removed from the vascular bundle present plastids of a fusiform shape (shown faintly in Pl. VI, Fig. 47), and thence one passes to cells with the familiar plastids of the bulk of the differentiated leaf-cells (Figs. 46 and 48). In the cells with thread-like archiplasts, pseudochondriosomes of the usual spherical type are present (Fig. 35). With Champy-Kull the archiplasts are also somewhat stained, but there is of course no ground for confusion because of the decided contrast in morphology.

Lateral buds in various stages of growth occur frequently, and afford excellent material for study. Fig. 33 is a cell near the base of an incipient lateral bud, very close to the main apical-point. The archiplasts only are demonstrated. In the lateral buds themselves, I did not obtain first-class Benda results, but so far as they went it was fairly clear that the general morphology of the plastidome was similar to that of the young root-tip cells. One very fine preparation was obtained of a lateral bud by the Champy-Kull method, in which practically every cell exhibited the pseudochondriome selectively stained. Fig. 56 shows the apical cell of this bud, and the same general picture was common to the other cells. The pseudochondriosomes present the customary spherical shape and are in no way different from the pseudochondriosomes in the other cells of *Equisetum*. The large cells in or around the base of a lateral bud are particularly good objects for study. In these cells the pseudochondriome may be stained alone or with an occasional plastid (Fig. 53), or both plastids and pseudochondriosomes may be stained together (Figs. 54 and 55). In any case, the demonstration of the pseudochondriome is usually the more definite. Again, the spherical shape, so typical of pseudochondriosomes, is predominant, and polymorphic forms are conspicuous by their absence.

Division phenomena were not particularly examined in cells from the growing points. However, in the younger leaf-cells, mitoses were common and presented features similar in many ways to the typical division figures in angiosperm meristem. Thus, in the distribution of the pseudochondriosomes, there is the same lack of any apparent connexion with the mitotic machinery, the granules being scattered at random throughout the cytoplasm (Fig. 43, and less clearly Pl. V, Fig. 31). In leaf-cells with young plastids, there is apparently some tendency towards the radiate arrangement at the spindle poles so characteristic of the archiplasts in angiosperms. In any event there is a clear-cut grouping of the plastids into two aggregates located at opposite poles of the dividing cell (Figs. 31 and 32, and less clearly Pl. VI, Fig. 43). Thus approximate equality of the pseudochondriome and plastidome in the daughter cells of *Equisetum* is arrived at by much the same route as in the case of angiosperms (Bowen, 3).

In the second of my Studies on plant protoplasm, I have discussed at length the grounds for believing that in angiosperms the osmiophilic platelets are a category of elements absolutely distinct from the chondriosome-like bodies. I have not gone into the matter with equal care in the case of *Equisetum*, since the general situation seemed so nearly identical. In many cases it was possible to impregnate both platelets and plastids together in the same cell, and in such cases the separate identity of the two elements is beyond dispute (Pl. VI, Figs. 45 and 52). It will be observed also that comparison of cells in the present paper prepared by the Champy-Kull method with corresponding cells from *Equisetum* in my first Study prepared by Kolatchev, indicate a clear separation between platelets and pseudochondriosomes. In general the platelets are markedly larger, less numerous, and with their own characteristic morphology. Furthermore, in old leaf-cells (Fig. 95 of Study I) the platelets present no new features, while the pseudochondriosomes may become more or less polymorphic (Fig. 48). It is difficult to see how platelets of a constant type could be produced as mere artifacts from elements of varying morphology. Guilliermond's (10) assertion that the platelets are merely altered plastids or pseudochondriosomes is accordingly not substantiated by the facts.

Discussion.

In the evidence presented in this paper there is one important gap, which could probably be closed by more extended technical trials. I refer to the conditions in the apical-cells. Fairly demonstrative cases of the plastidome in the apical-cell of the root-tip and of the pseudochondriome in the apical-cell of a (lateral) growing-point were obtained (Pl. V, Fig. 7 and Pl. VI, Fig. 56); but first-class preparations of the complementary type are in each case lacking. It is, nevertheless, a practical certainty that the pseudochondriome, for example, is essentially the same in the apical-cell of the root-tip as in that of the growing-point. For all practical purposes the two kinds of apical cells are probably cytologically identical. Throughout most of the other kinds of cells, the separate identity of pseudochondriome and plastidome has been directly established. As a result of these observations it is, therefore, probable that in *Equisetum* (Pteridophyta) the cytoplasmic structures are the same as in the angiosperms. This applies not merely to the classes of bodies which can be differentiated, but to their staining capacities, shape, structure, division phenomena, and function. The osmiophilic platelets and the pseudochondriosomes present in both groups of plants, identical characteristics, and there can be no doubt that, on one hand, the pseudochondriosomes in the angiosperm cell are homologous to those in the pteridophyte cell, and that, on the other hand, a similar homology exists in respect to the platelets. So also in the case of the plastidome, although there is more variability in the shape of the archiplasts

(just as occurs in different plants within the angiosperm group), nevertheless the exact equivalence of the plastidome in Pteridophyta and Spermatophyta is thoroughly demonstrated.

In respect, therefore, to the chondriosome-like bodies of *Equisetum*, my conclusion, long since anticipated for purposes of description, is that these bodies as a group must be separated into two distinct categories. One class, forming the plastidome, is involved solely with the phenomenon of plastid formation; the other class, the pseudochondriome, has nothing whatever to do with this particular operation. In meristem cells, the elements of the plastidome are characterized by a varied shape tending to be distinctly elongate; while the pseudochondriosomes are typically spherical. No evidence of a conclusive nature can be adduced from observation of this material which may be considered as actual proof of the mitochondrial nature of either (or both) of these constituents. The chondriosomal homologies of the 'chondriosome-like' bodies in the plant cell must positively be left for a future decision which further data will probably permit.

The chondriosome-like bodies in the pteridophyte cell have not been very extensively studied by other workers. Certain facts, such as the unique condition of the plastidome in *Selaginella*, have of course been known for some time. The amazing behaviour of the plastidome during division of the spore mother-cells has been studied by Fitting (8) in *Isoetes*, and by Marquette (14) in *Marsilia*. The behaviour of the plastids in mitosis of leaf-cells in *Isoetes* has also been reported by Marquette (13). Schimper (22) found chlorophyll-bearing granules in the apical cell of *Azolla*, and also examined the spores of several ferns and *Equisetum* for chlorophyll-bearing bodies. He (23) also studied the chromoplasts in the fertile shoots of *Equisetum arvense*. The history of the plastidome during sporogenesis has been followed by Sapĕhin (20) in *Lycopodium* and *Selaginella*. In most of these cases, the presence of unusually definite plastids made the observations possible in the first place, and they throw no particular light on the more general problem with which this paper is particularly engaged.

The chondriosome-like bodies in Pteridophyta have been further examined in part by Pensa (17, 18), Lewitsky (11), and Senjaninova (24). Pensa himself did not attempt to establish any definite homologies with animal chondriosomes. He observed only the plastidome (in *Scolopendrium* and *Aspidium*), demonstrated in the most selective and clear-cut manner by silver impregnation. Lewitsky, working on the spore mother-cell divisions in *Equisetum*, and Senjaninova on sporogenesis in *Nephrodium* (= *Aspidium*) did not differentiate between plastidome and pseudochondriome. All the elements shown in their preparations were simply called chondriosomes. There is some reason to believe that Senjaninova at

least demonstrated principally the plastidome. In any event no effort was made by either worker to analyse the possibilities of the situation. In view of the other work now to be noted, it seems probable that a more adequate technical approach might reveal conditions homologous to those which I have found in the sporophyte of *Equisetum*.

A study of the cytoplasmic situation in Pteridophyta from a point of view more like that adopted in my own work, has been made only by Mottier (16), Dangeard (4), and particularly Emberger (6, 7). These observations cover the root-tip of *Adiantum* (Mottier); various tissues of *Selaginella* (Dangeard); and a complete survey of various species of *Selaginella*, *Lycopodium*, and *Equisetum*, together with a number of ferns (Emberger). The findings deal of course only with the chondriosome-like bodies, since the occurrence of osmiophilic platelets was unknown.

Mottier's results on the root-tip of *Adiantum* are very similar to my findings in *Equisetum*. He finds throughout the young part of the root two kinds of chondriosome-like bodies. One of these consists of many small, spherical granules, sometimes slightly elongated, and clearly the same as the pseudochondriome. The other kind of elements constitute the plastidome as clearly revealed by their relation to leucoplast formation. The development of leucoplasts in the root-cap, periblem, and dermatogen, is quite like the situation in *Equisetum*. Even more striking is the failure to develop leucoplasts in the early plerome cylinder, and the appearance instead of much elongated archiplasts. This is in turn similar to the condition in *Equisetum*. The apical-cell is apparently a better object for study than in *Equisetum*, for here the archiplasts have the appearance of small leucoplasts, and the separation of plastidome and pseudochondriome is relatively easy. In the apical-cell of *Equisetum* I did not find any indication of the presence of leucoplasts, or of archiplasts with a structure at all reminiscent of leucoplasts. Emberger (6), however, reports the presence of small leucoplasts in the apical-cell of *Equisetum*, and starch is certainly present in the apical-cell of some ferns (Emberger 6). Since the leucoplasts in *Equisetum*, as described by Emberger, are in any event very small, it is possible that their occurrence might be subject to variation according to the conditions in a given apical-cell. In general, therefore, Mottier's results and his conclusions from his results accord with mine, although, judging from the figures, I was able to effect a sharper demonstration of the pseudochondriosomes in cases where successful results were obtained.

Dangeard (4) examined cells from various regions of *Selaginella*, both in living and stained preparations. The conditions in many kinds of cells in *Selaginella* are admirably suited for a study of the chondriosome-like bodies, because in many cases, and particularly in meristematic tissues (including the root-tip), there is but one plastid present. The plastidome is thus delimited with the most astonishing clarity, and a more favourable

setting in which to study the separate identity of the pseudochondriome, could not be imagined. Dangeard finds the pseudochondriosomes (at that time he termed them 'microsomes') in their usual simple, spherical form, exactly like the type which I have constantly observed in plant cells. He considers these granular bodies as absolutely distinct from the plastidome, and thus again my results conform both in essential substance and in conclusions with those of Dangeard. Dangeard, it is to be noted, rejects the chondriosome terminology altogether, while Mottier is inclined to apply the term chondriosome to the granular elements (pseudochondriosomes).

The most extended study of the chondriosome-like bodies in the Pteridophyta has been carried out by Emberger (6, 7) on representatives of several of the main subdivisions of the group. He examined cells of all kinds in both sporophyte and gametophyte generations, and by *intra vitam* methods as well as fixation and staining (especially Regaud-Fe-haematoxylin). He is the only other observer who has studied the root and growing-point of *Equisetum*. His very extended studies of pteridophytes need not here be reviewed in detail, and it will suffice to point out that his conclusions are in essential agreement with those of Mottier, Dangeard, and myself. We are all agreed that there are two types of elements in the cytoplasm of pteridophytes, one associated with plastid formation, the other not so associated. The distinct nature of these two categories of bodies is further emphasized by the results of Pensa (17, 18), whose technique permitted the extraordinarily precise demonstration of the plastidome, while the pseudochondriosomes remained absolutely invisible. As to the chondriosomal nature of any of these bodies, Pensa and myself have left the matter undecided, at least for the present, Mottier apparently accepts only the pseudochondriosomes as the representative of the chondriome, Dangeard rejects the whole chondriosome conception, and Emberger considers all of the bodies as chondriosomes, though of two fundamentally distinct varieties. This last interpretation does not seem to me in accord with the observed facts, and I cannot accept it in this case any more than in that of the angiosperms, where I have discussed the matter at length (Bowen 3). Even Emberger's own printed results do not support the supposed microchemical identity of all the chondriosome-like bodies in meristem cells, as required by Guilliermond's original statement of the theory of a duplex chondriome in plants. Thus Emberger (7) could not demonstrate the pseudochondriosomes in the vegetative point of *Lycopodium*, and he found other cases where inequalities in staining capacity certainly do not point towards a common microchemical basis. However, these differences of opinion apart, the fact stands out that the pteridophyte cell, like the angiosperm cell, contains two distinct categories of bodies—plastidome and pseudochondriome, each category in all cases possessing remarkably similar characters of every kind.

I am nevertheless constrained to add that while my findings agree in the main with those of Emberger, in many cases the pseudochondriosomes of my preparations do not look at all like those which he figures. For example, the pseudochondriome of his Fig. 2, Pl. VIII (6) does not appear at all like my Pl. V, Fig. 23 of the same kind of cell. Why there should be such an extraordinary difference is difficult to explain, for in living cells generally, the pseudochondriosomes are sharply definable and are usually spherical, sometimes slightly elongate, very much as I have drawn them in fixed preparations. I assume that differences in technique are the chief factor involved, but it is, to say the least, curious that preparations so different in the appearance of their details, lead to conclusions which in many respects are in essential agreement.

Summary.

1. A study, in *Equisetum arvense*, of the cytoplasmic components which have the general characteristics of animal chondriosomes, has shown that these bodies are of two different categories.

2. One type forms the *plastidome*, a collection of elements which, in young meristem cells, are of varied shapes but are predominantly elongate. From these bodies leucoplasts or chloroplasts are gradually differentiated.

3. The other type forms the *pseudochondriome*, so-called because of its many resemblances to the animal chondriome. The elements of this kind are typically small spheres, tending towards a more polymorphic development in old cells. These bodies are chiefly characterized by their apparent lack of any function which can express itself in visible phenomena.

4. So far as this study can show, the plastidome and pseudochondriome have absolutely nothing to do with each other, and represent two independent categories of cytoplasmic bodies. This is proved by constant differences in staining capacity, morphology, structure, and functional behaviour.

5. These conclusions are in the main confirmatory of the previous findings of Pensa, Mottier, Dangeard, and Emberger. They do not agree with the recent finding of Senjaninova, who finds in pteridophyte cells only one type of cytoplasmic bodies—so-called chondriosomes.

6. No evidence has yet been obtained in *Equisetum* which would at present permit the final identification of any or all of the chondriosome-like bodies as the actual homologues of the chondriosomes in animal cells. Such comparisons must await further data of a more critical nature.

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EXPLANATION OF PLATES V AND VI.

Illustrating Dr. Robert H. Bowen's paper on Chondriosome-like Bodies.

All of the figures, with a few exceptions noted below, have been outlined as far as possible with the camera lucida at an initial enlargement of approximately 1,675 diameters, and subsequently completed free-hand. In reproducing, the figures have been reduced uniformly to an enlargement of approximately 1,250 diameters. In the case of Figs. 6, 20, 24, 47, and 48, the initial enlargement was approximately 2,775 diameters, with reproduction at approximately 2,100 diameters. Figures from root-tips are printed with that side of the cell at the top which was originally directed away from the growing tip of the root; similarly, cells from growing-points are printed with that side towards the top which was originally directed towards the free growing tip. The method employed in the preparation of the original object is appended to the explanation of each figure, details being given according to the formulae explained on page 706 of my first Study on plant protoplasm (Bowen, 1928) and extended in the second Study (Bowen, 1929).

EXPLANATION OF FIGURES.

PLATE V.

Figures 1 to 24 inclusive are from root-tips of *Equisetum arvense*; the remaining figures are from young growing-points of the same species.

Fig. 1. Root-cap; early stage near apical cell. E-Champy-Benda.

Figs. 2–4. Root-cap; stages in the development of leucoplasts. E-Champy-Benda.

Fig. 5. Old root-cap cell. E-Mottier-Benda.

Fig. 6. Examples of developing leucoplasts from root-cap cells. E-Champy-Benda.

Fig. 7. Apical cell. E-Mottier-Benda.

Fig. 8. Cell just a little above the apical cell. E-Mottier-Benda.

Fig. 9. Plerome-periblem border near apical cell. E-Mottier-Benda.

Fig. 10. Cells from early outer plerome. E-Mottier-Benda.

Fig. 11. Plerome; slightly older than preceding figure. E-Mottier-Benda.

Figs. 12 and 13. Large cells from centre of plerome. Fig. 13 is at a higher level than Fig. 12. E-Mottier-Benda.

Fig. 14. Telophase; cell like that of Fig. 13. E-Mottier-Benda.

Fig. 15. Dermatogen; very close to apical cell. E-Champy-Benda.

Fig. 16. Dermatogen; beginning of leucoplast development. E-Mottier-Benda.

Fig. 17. Dermatogen; early stage in leucoplast formation. E-Mottier-Benda.

Fig. 18. Outer layer of periblem; prior to leucoplast formation. E-Mottier-Benda.

Fig. 19. Periblem; early leucoplasts. E-Mottier-Benda.

Fig. 20. Examples of leucoplasts from the periblem. E-Meves-Benda.

Fig. 21. Old root-cap. E-Champy-Kull.

Fig. 22. Outermost layer of plerome. E-Champy-Kull.

Fig. 23. Inner layer of periblem. E-Champy-Kull.

Fig. 24. Cytoplasmic fragment from a cell like that of Fig. 23, showing a single leucoplast and several pseudochondriosomes. E-Champy-Kull.

Figs. 25 and 26. Cells from outermost layer of a leaf near its base. E-Mottier-Benda.

Fig. 27. Like preceding figures, but an older stage farther up the leaf, and showing the transition towards plastids. E-Mottier-Benda.

Fig. 28. Like preceding figure; plastids developing. E-Mottier-Benda.

Fig. 29. Cell like preceding figures, but from an inner layer of the leaf. Plastids developed. E-Mottier-Benda.

Fig. 30. Cell much like that of Fig. 29, but from an area of larger cells. E-Champy-Fe-haematoxylin.

Fig. 31. Cell like that of Fig. 30; late telophase. E-Champy-Fe-haematoxylin.

Fig. 32. Cells much like that of Fig. 30; resting and metaphase stages. E-Champy-Fe-haematoxylin.

PLATE VI.

All the figures are from young growing-points of *Equisetum arvense.*

Fig. 33. Cell near the base of a leaf-bud arising close to the tip of the growing-point. E-Mottier-Benda.

Fig. 34. Cell from the region along a developing vascular bundle in a leaf. E-Mottier-Benda.

Fig. 35. Portion of a cell like the preceding figure. E-Champy-Kull.

Fig. 36. Leaf-cell similar to that of Fig. 25. E-Champy-Kull.

Fig. 37. Leaf-cell like that of Fig. 36, showing also some of the proplastids. E-Champy-Kull.

Fig. 38. Leaf-cell somewhat older than that of Fig. 37, with early steps in transformation of proplastids into definitive plastids. E-Champy-Kull.

Fig. 39. Cell from a leaf-base, with developing plastids. E-Champy-Kull.

Figs. 40–42. Leaf-cells similar to those of Figs. 29–32. The three cells show various steps in the development of the large vacuolar spaces of old leaf-cells. E-Champy-Kull.

Fig. 43. Cell like those of Figs. 40 and 41; telophase. E-Champy-Kull.

Fig. 44. Portion of an old leaf-cell. E-Champy-Kull.

Fig. 45. Same as Fig. 44. K–E (1 %–40° : 35°) 8.

Fig. 46. Old leaf-cell; tangential fragment. E-Meves-Kull.

Fig. 47. Portion of the cytoplasm from a leaf-cell along a vascular bundle. E-Champy-Kull.

Fig. 48. Portion of the cytoplasm from an old leaf-cell. E-Champy-Kull.

Fig. 49. Guard-cell in a leaf. The nucleus lies in an adjacent section. The plastidome is not demonstrated. E-Champy-Kull.

Fig. 50. Young leaf-cell. W2¼–E (2 %–25°) 8.

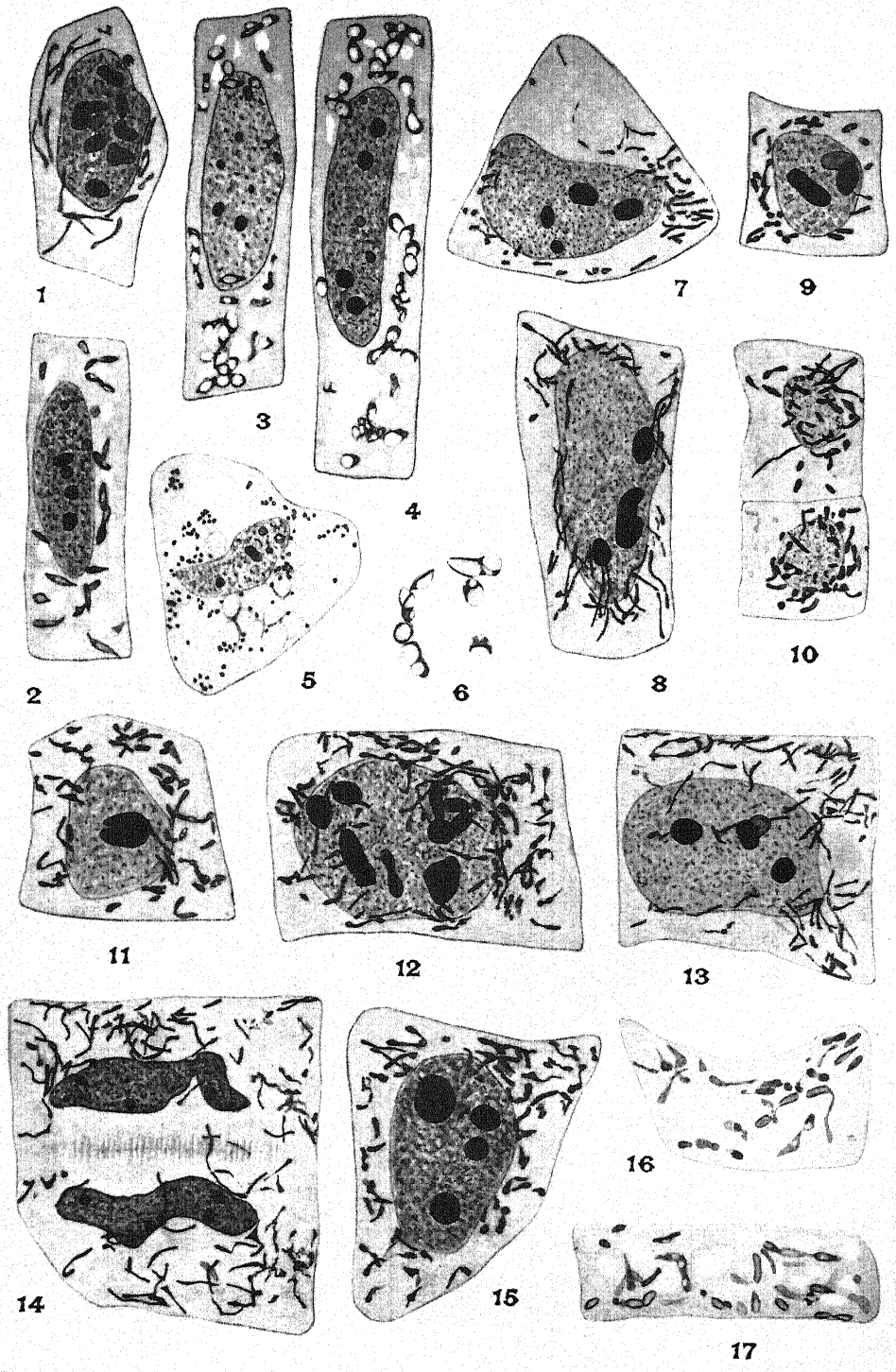
Fig. 51. Cell from the base of a leaf (or the main stem), on the periphery of the base of a new lateral bud. E-Champy-Kull.

Fig. 52. Cell in much the same position as that of Fig. 51. K–E(1 %–40° : 35°) 8.

Figs. 53 and 54. Cells from the area at the base of a lateral bud. E-Champy-Kull.

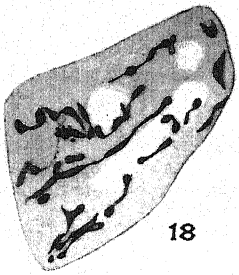
Fig. 55. Cell much like those of Figs. 53 and 54, but more removed from the bud-base, and close to a vascular bundle. E-Champy-Kull.

Fig. 56. Apical cell from a lateral bud. E-Champy-Kull.



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BOWEN — CHONDRIOSOME-LIKE BODIES.



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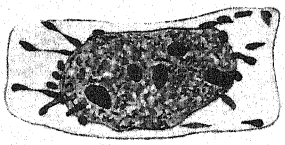
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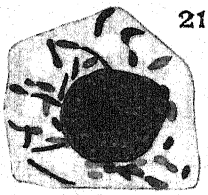
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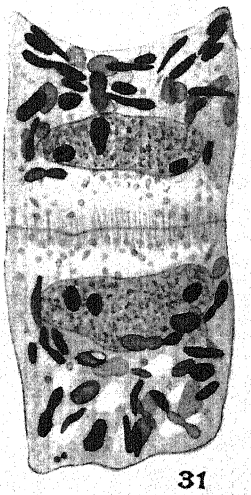
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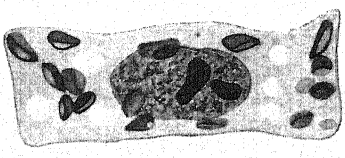
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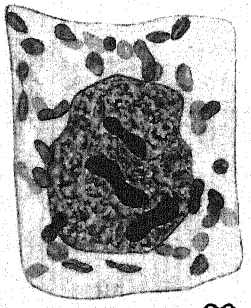
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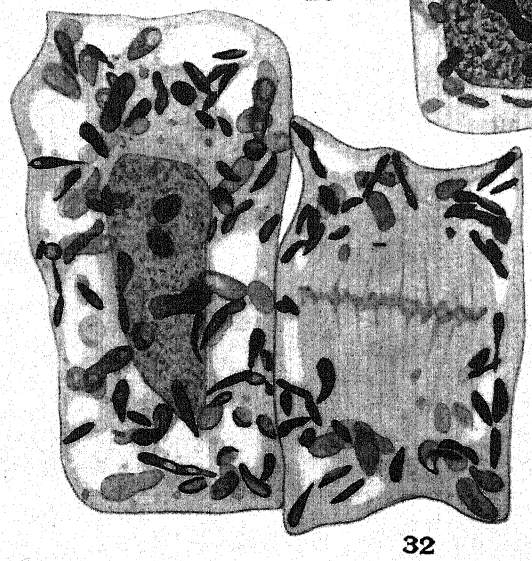
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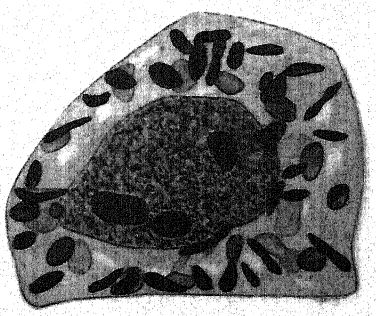
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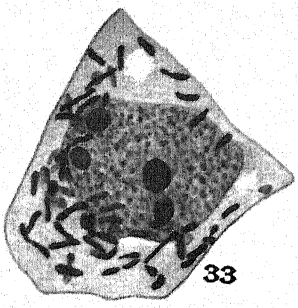


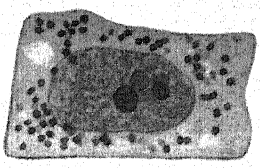
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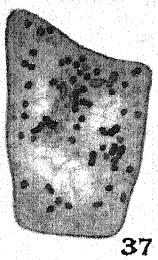


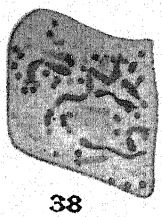
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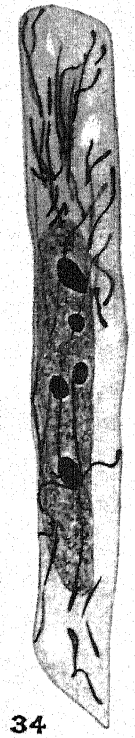
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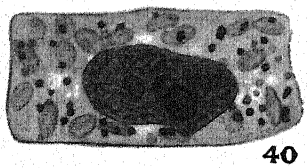

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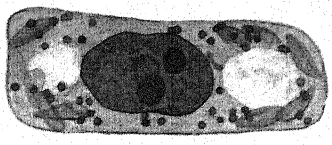

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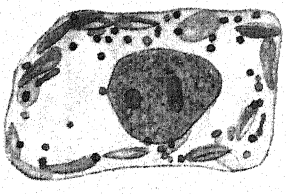

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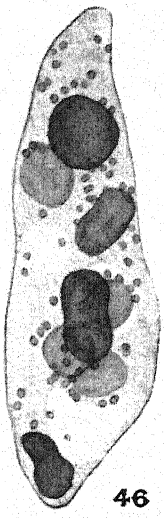

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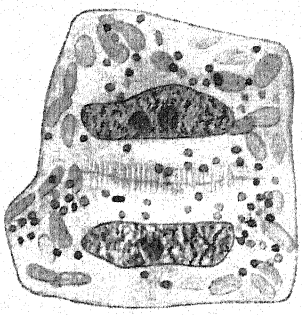

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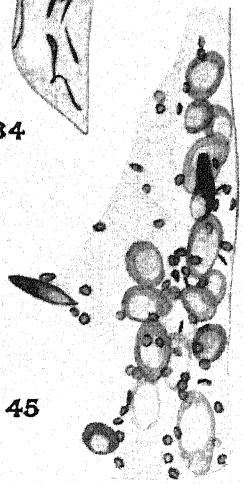

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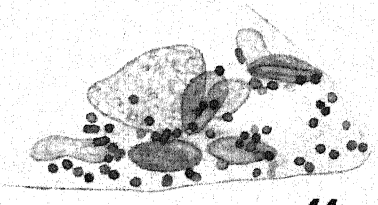

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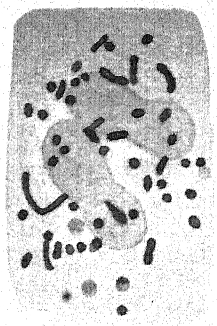

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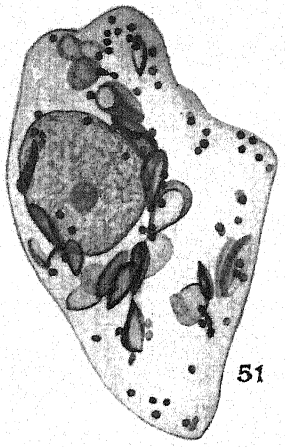

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R. H. Bowen del.

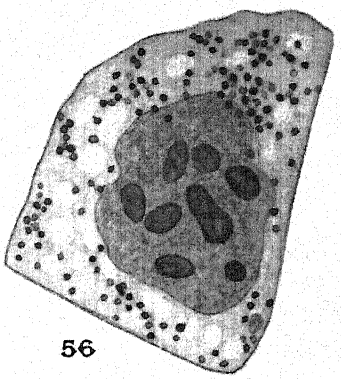
BOWEN — CHONDRIOSOME-LIKE BODIES.



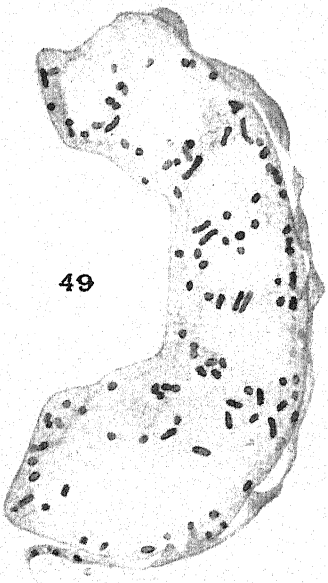
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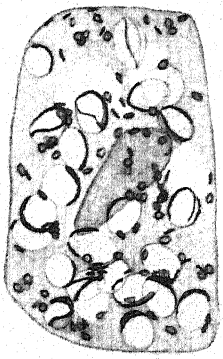
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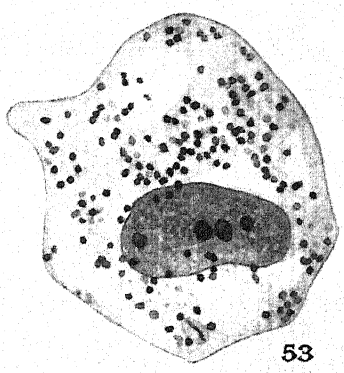
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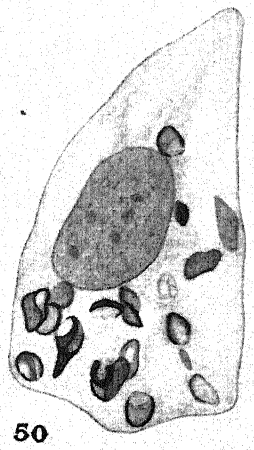
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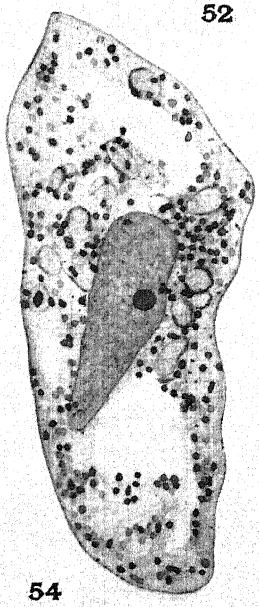
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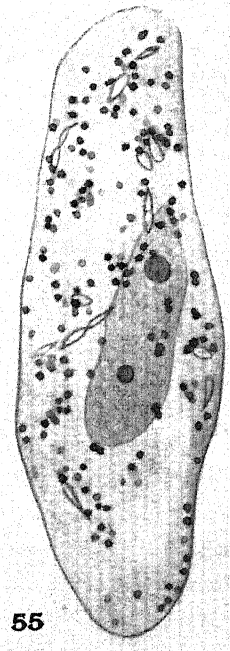
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Huth, London.

The Interaction of Factors in the Growth of Lemna.

II. Technique for the Estimation of Dry Weight.

BY

M. THET SU

AND

ERIC ASHBY.

(From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London).

With two Figures in the Text.

BEFORE progress could be made in the of growth studies of *Lemna* which are being carried out in this laboratory, it was necessary to evolve a rapid and reliable method for comparative dry-weight estimations. The method described here was briefly outlined in the first paper of this series (1). Since difficulty is often experienced in obtaining reliable comparative values for the dry weights of small quantities of tissue, a description of the method should be of value.

The aim of the method is to give as complete a drying as possible in the shortest time and without risk of decomposition. Drying of biological material at high temperatures is known to be unsatisfactory (2). Owing to the time it requires, drying at room temperature is impracticable. After a number of trials it was found that when the samples of *Lemna* plants were dried for half an hour at 50° C. in a vacuum of 5 mm. pressure of mercury, consistant results were obtained, so long as the same routine method was followed for all weighings.

The drying apparatus, Fig. 1, consists of a large desiccator containing concentrated sulphuric acid. On a glass tripod in the desiccator stands a resistance mat, *a*, the leads to which pass in through a rubber cork in the side of the desiccator. The resistance mat is in series with a rheostat, and the resistance is so adjusted that when the current is on, the temperature of the mat remains at 50° C. A thermometer lies permanently on the resistance mat. The desiccator communicates with the air through two taps, *b* and *c*; the former leads to a manometer and a Geryk suction pump, the latter to a wash bottle of sulphuric acid, through which dry air can be

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drawn into the desiccator. The rubber corks were sealed with collodion containing about 5 per cent. castor oil. A mixture of resin cerate and vaseline was used between the cover and the body of the desiccator.

The plants are dried and weighed in platinum boats contained in light glass phials, Fig. 2. The stoppers of the phials are ground; and each

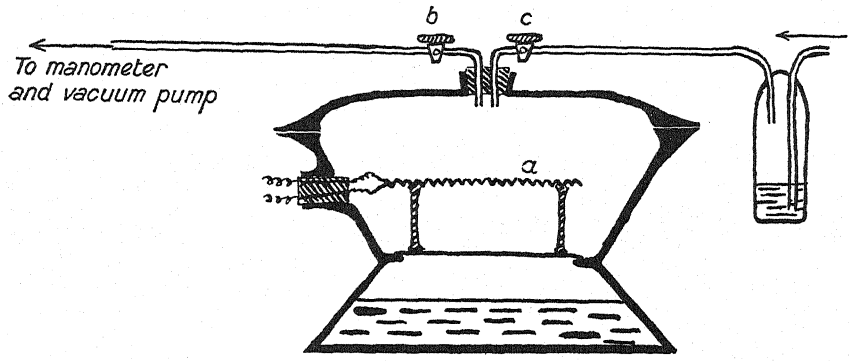


FIG. 1.

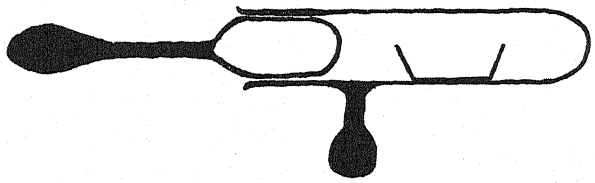


FIG. 2. Twice natural size.

phial with its platinum boat is counterpoised against a similar phial weighted with pieces of lead and aluminium. The use of ground stoppers avoids to a large extent the absorption of moisture. Both phial and stopper have flattened glass handles to facilitate lifting them on and off the balance.

The weighings were carried out on a Bunge micro-balance. For details of the technique of weighing, reference should be made to Pregl's monograph (3).

PROCEDURE.

For the sake of completeness, the description of the procedure given in (1) is repeated.

In a normal experiment, four samples are dried at the same time and weighed successively. The procedure is as follows: The platinum boats are boiled in nitric acid, washed and dried, and put into their respective phials. The phials are counterpoised. The four samples of plants are then packed into the four boats, and the boats put back into the phials. The

four phials and their complements are then laid, with their stoppers out, on the resistance mat in the desiccator. The desiccator is exhausted until the manometer shows a pressure of 5 mm. mercury. Then the current is turned on. The temperature of the mat rises rapidly to 50° C. and remains at this temperature. After half an hour at 50° C. the mat is allowed to cool. When the thermometer lying on the mat indicates about 25° C., dry air is admitted through the tap *c*. The cover is moved about three inches to admit a pair of long forceps into the desiccator, and with these the stoppers are pushed into their respective phials. The phials are then weighed, one being taken out of the desiccator at a time. The desiccator is closed during the weighings.

TESTS.

Before the method was adopted, certain tests were carried out.

1. *The effect of drying for longer periods than half an hour.* Twenty fronds of *Lemna* were dried for half an hour at 50° C., six successive times, and weighed after each half hour. The results of two experiments, at 5 mm. mercury pressure, are given:

		Expt. 1.	Expt. 2.
1st	drying	0·969 mg.	0·825 mg.
2nd	,,	0·967 ,,	0·822 ,,
3rd	,,	0·970 ,,	0·826 ,,
4th	,,	0·962 ,,	0·819 ,,
5th	,,	0·967 ,,	0·824 ,,
6th	,,	—	0·824 ,,

There is obviously no significant change in weight after the first drying, and it was concluded that a sufficiently good 'end point' was reached after half an hour.

2. It had to be determined whether the leaving of samples in the desiccator after air had been admitted affects the values of their dry weights. Four samples were dried four times in succession under the standard conditions, and weighed in the order *a-b-c-d* after the first drying, *b-c-d-a* after the second, and so on. The figures give no evidence that weights of samples left in the desiccator after opening are affected.

	Order of Weighing.			
	a–b–c–d.	b–c–d–a.	c–d–a–b.	d–a–b–c.
Sample 1	2·319 mg.	2·324 mg.	2·326 mg.	2·327 mg.
,, 2	2·579 ,,	2·578 ,,	2·583 ,,	2·565 ,,
,, 3	2·403 ,,	2·397 ,,	2·408 ,,	2·418 ,,
,, 4	2·392 ,,	2·383 ,,	2·395 ,,	2·380 ,,

3. *The absorption of moisture by the dried tissue.* When samples are weighed in phials whose stoppers are not ground, there is a marked absorption of moisture, and it is impossible to obtain a consistent value for the

dry weight. When ground stoppers are used, no increase in weight is observed for five or six minutes during weighing, after which time the weight gradually rises. Moreover, if the weighing is performed within five minutes of removal from the desiccator, consistent results are obtained for the dry weight per unit area of both large and small samples. Therefore any absorption of moisture between the time of removal from the desiccator and weighing on the balance does not significantly affect the comparative value of the results. Even high humidities do not diminish the accuracy: at a temperature of 23° C. with an open bowl of water in the outer balance case no change was observed in three successive weighings made within six minutes.

In estimating the probable error of the dry weight of a single sample, the errors of this method of drying are automatically included in the sampling error. It is impossible, therefore, to evaluate the error of the method alone. That this error is very small may be deduced from the fact that the probable error of the *ratio* of carbon per unit dry weight (determined in 1), is only 0·3 per cent.

It is thought that a method such as this, dealing with quantities as small as 0·5 milligrams of dry weight of material, might be of wider interest.

In conclusion the authors wish to thank Professor V. H. Blackman for helpful criticism, and Mr. B. D. Bolas for his kind co-operation.

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The Interaction of Factors in the Growth of Lemna.

III. The Interrelationship of Duration and Intensity of Light.

BY

ERIC ASHBY.

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With Plate VII and eight Figures in the Text.

1. Introduction.

FROM the literature of so-called photoperiodism one fact stands out clearly: that the influence of light upon growth processes depends on the length of the daily period of illumination, quite apart from the intensity of the light employed. The relevant literature has been reviewed by Kellerman (3). The nature of this influence of 'length of day' is obscure; most investigations have been concerned only with flowering, nor have they been carried out under carefully controlled conditions.

In this paper are set out the results of experiments upon the interrelation of intensity of light and daily period of illumination on the growth processes of *Lemna minor*. The technique for the minute analysis of growth, described in the earlier papers of this series (1 and 2), is well suited to such an investigation. The work is still in progress, but certain relationships have already been established from the experiments to be described. It is hoped that in the course of further work some light may be thrown upon the mechanism of the effect of 'length of day'.

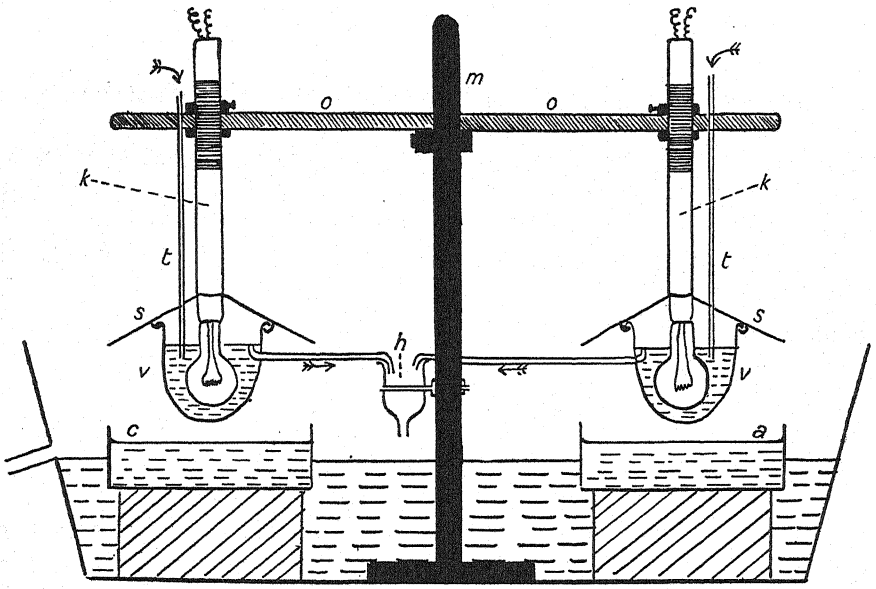
2. Description of Apparatus.

The apparatus (Text-figs. 1 and 2) in which the colonies are grown is a modification of that described in the first paper of this series (1). Four growth chambers, *a*, *b*, *c*, *d*, are connected in series by glass tubes. The culture solution is aerated and circulated through the growth chambers by a suction pump, *e*, in the manner described (1). One complete circulation of the solution takes about forty-five minutes. The whole system of chambers is immersed in a large water bath, heated from below by carbon filament lamps, and kept at a constant temperature by a thermo-regulator, *g*. Contact in this regulator is made between platinum and mercury in an

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atmosphere of hydrogen, so that there is no fouling of the surface. The temperature of the bath was found to remain almost constant, variations greater than 1/10° C. being rare. It is thus ensured that the four experimental colonies of *Lemna* plants shall grow in the same culture solution, and at the same temperature.

Each growth chamber is illuminated by one gas-filled lamp, varying in different experiments from 50 to 200 watts, enclosed in an open round-

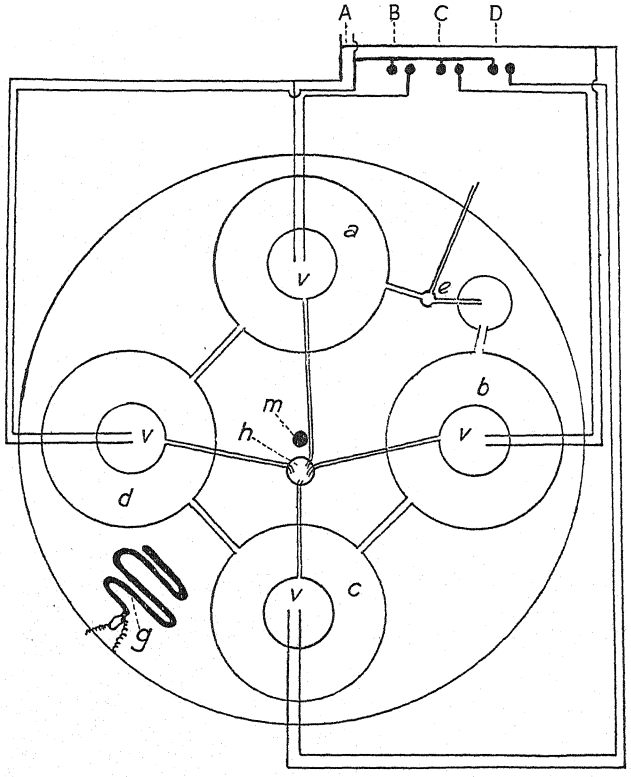


TEXT-FIG. 1. The growth chambers: elevation. For explanation of lettering, see text.

bottomed glass vessel *v*. Between the lamp and the vessel is a jacket of flowing water which is so efficient that even with 200 watt lamps the surface of the enclosing vessel remains cold. Each vessel is secured by a ring of copper wire to the lamp reflectors. Water enters the water-jacket through the top of the reflector, *t*, and flows to the drain, *h*, through a tube in the side of the vessel. At first it was found that water vapour condensed on the bottom of the water screens, making the field of light uneven. This was overcome by warming the water before it entered the water-jackets, so that the temperature of each water-jacket was slightly higher than that of the culture solution below it. The water was warmed by passing it through a spiral of metal tubing wound round a long carbon filament lamp.

The long lamp holders, *k*, are screwed into four horizontal arms of wood, *o*, mounted on a central stand, *m*. The distance of the lamps from the culture solution is adjusted by moving the four arms up and down the

central stand, and individual adjustments can be made by means of the nuts which secure the lamp holders to the four horizontal arms. In spite of air bubbles, which sometimes form in the water-jacket, the lamps give an even field over about 7 square inches. Since the colonies of plants do not remain entirely stationary in the stream of solution, the effects of any small

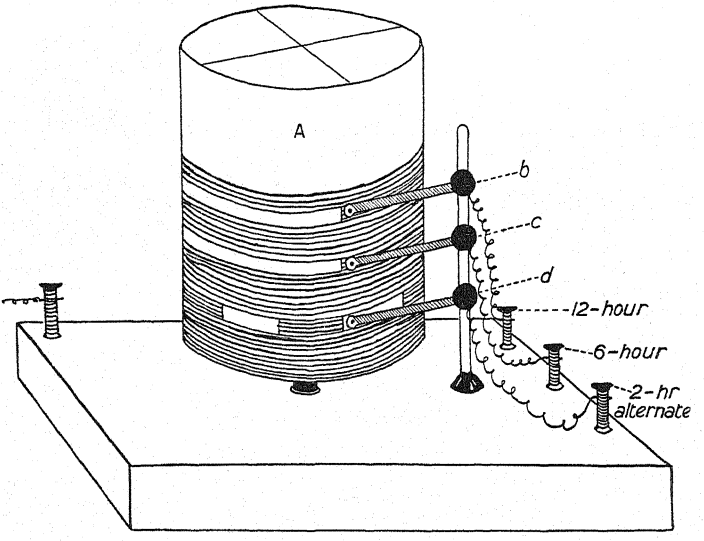


TEXT-FIG. 2. The growth chambers: plan. Details of the wiring are shown. At B, C, and D are the terminals of the three relays, and at A the leads to the mains. (B = terminals to 2-hour alternate relay; C = terminals to 6-hour relay; D = terminals to 12-hour relay) According to the wiring in the diagram, chamber *a* is receiving the 2-hour alternate period of illumination; *b* is receiving the 12-hour; *c* the 6-hour, and *d* the continuous periods.

inequalities in light intensity are annulled. The temperature difference between the culture solution, when the lights are on and off, never exceeds 0·3° C.

Of the four lamps, one over each chamber, one runs continuously, a second for twelve hours, a third for alternating two-hour periods, and a fourth for six hours, out of every twenty-four. These 'lengths of day' will be referred to as 'continuous', '12-hour', '2-hour alternate', and '6-hour' periods respectively. The lamps are turned on and off by means of a time switch (Text-fig. 3). This switch consists of a metal drum, A,

revolving once every twenty-four hours ; covered with celluloid, from which strips, corresponding to the various periods of darkness, are cut away to expose the metal surface of the drum. Three spring contacts, *b*, *c*, and *d*, lie against the surface, and these make contact with the exposed parts of the drum as it rotates, and close a four-volt circuit. The current lifts a relay and the corresponding lamp is turned off. The lamp connexions are



TEXT-FIG. 3. Time switch. For explanation of lettering, see text.

labelled, and by interchanging them the different light periods can be made to operate on any of the four lamps.

All extraneous light is excluded from the growth chambers by enamelling them, on the outside, a dull black ; the connecting tubes and the aerating tubes are also enamelled in the same way. Each growth chamber is covered with a closely fitting hood of black silk, secured to the rim of the chamber by an elastic band.

The colonies of *Lemna* are grown within the floating frames of glass tubing, described in (1).

The culture solution employed is that recommended by Clark (4). About 40 litres are prepared before an experiment, and are stored in a large earthenware container, the glaze of which is free from lead.

3. EXPERIMENTAL PROCEDURE.

Before an experiment begins, the population of *Lemna* is kept in one of the growth chambers for about four days, under the light intensity to be employed, and at the 12-hour light period. The lights are all adjusted to give the same intensity at the surface of the solution. Then, from the

uniform population, four colonies of about 120 fronds are counted, one into each of the four growth chambers. From the remainder of the population four samples are taken and the area and dry weight of these is found. This constitutes the 'preliminary sampling'. The number of fronds and plants in each colony is counted every day. On the first day of 'routine sampling', two samples are taken from the continuous colony, and two from the 12-hour colony; on the second day, two samples from the 2-hour alternate colony, and two from the 6-hour colony, and so on. In any one colony, therefore, a value is obtained for area and dry weight every other day.

Every two days the solution is changed, and the whole apparatus cleaned out with absorbent cotton wool.

The daily routine procedure is as follows: 1. Temperature reading. 2. Testing of the pH with methyl red. 3. Measurement of the light intensities. A Holophane lumeter reading in foot candles is used for this purpose. A small square of matt-surface opal glass which rests on the floating frame in the culture solution is used as a test surface. This square was standardized against the standard surface provided with the lumeter. 4. Counting the colonies. The plants are transferred from the growth chambers into four beakers containing culture solution, and the required samples are removed. The colonies are then counted, one plant at a time, and placed in a growth chamber different from that previously occupied. This cancels any local effects of the four growth chambers. 5. The connexions to the lamps are changed to different relays, so that each colony may still have its proper light period. 6. Finally, the area and dry weight of the samples are found as described in the earlier papers of the series (1 and 2). The carbon content was not estimated.

4. Statement of Results.

Four experiments were carried out under the same routine conditions.

Date.	Expt.	Light in Ft. Candles.	Periods.			
Sept. 1928	1	350	Continuous	12-hr.	2-hr. alt.	6-hr.
June 1928	2	700	Continuous	12-hr.	2-hr. alt.	6-hr.
Sept. 1928	2*a*	700	Continuous	12-hr.	—	—
Nov. 1928	3	1400	Continuous	12-hr.	—	6-hr.
		320	Continuous	—	—	—

In all four experiments the temperature remained at 25° C., and the pH of the solution at 4·8. Ordinary laboratory air, with a CO_2 concentration of about four parts in 10,000, was passed over the colonies. In Experiments 1 and 2 no difference was observed between the '12-hour' and the

'2-hour alternate' colonies. For this reason the 2-hour alternate light period was omitted from Experiment 3, and replaced by a continuous period at 320 foot candles, an intensity slightly lower than that of Experiment 1. In September 1928, immediately after Experiment 1, the light intensity was raised to 700 foot candles, so that the experiment made in June might be repeated. This checking experiment, together with that at 320 foot candles in November, was to ascertain if experiments carried out successively over a number of months gave similar results. Experiments 1 and 2 lasted 10 days, Experiment 3 lasted 9 days, and Experiment 2 *a*, 5 days.

In setting out the results, the actual frond numbers counted have been reduced in each experiment to the initial value of 100, so that one experiment should be easily comparable with another. The figures given *include* the allowance made for samples removed from the colonies.

5. Experiment 1.

Light: 350 foot candles, 100 watt lamps; begun 19 September, 1928.

For three days before the experiment began the plants were kept under a light intensity of 350 foot candles and a 12-hour period. Owing to the slow rate of multiplication of the colony under the 6-hour period, it was not possible to sample so frequently as in the other experiments. On the ninth day the relay regulating the thermostat failed, and the temperature fell to 18° C. for some hours; values after the ninth day have accordingly been rejected. Throughout the experiment the plants were green and apparently quite healthy. The results are shown in Table I.

The values for increase in frond number, area, and dry weight, at continuous, 12-hour, and 2-hour alternate periods fall closely upon exponential curves. By the method of least squares (or, where the agreement is close, by graphical methods), equations of the form $x = Ce^{rt}$ have been fitted to the values for frond number (Text-fig. 4), and for dry weight (Text-fig. 7). The calculated and the experimental figures for frond number are set out in Table II.

The exponential curves are typical of unrestricted growth. It must be concluded, therefore, that although the light intensity is low, the plants are not 'living above their income' at the continuous and 12-hour and 2-hour alternate periods. The close agreement between the growth at the 12-hour and 2-hour alternate periods indicates that for the duration of the experiment the action of light at these two periods was the same.

The growth at the 6-hour period gives a totally different curve. It is markedly rhythmic, it alternately rises and falls, and its general trend is in a straight line, though the experiment was not continued for a long enough period to discover whether growth was ultimately suspended. The unrestricted

TABLE I (350 foot Candles).

	Continuous.				12-hour.			
Day	Plants.	Fronds.	Total Area (P.E. ±2·4 %.)	Total Dry Weight (P.E. ±2·1 %). mgm.	Plants.	Fronds.	Total Area (P.E. ±2·4 %).	Total Dry Weight (P.E. ±2·1%). mgm.
0	34	100	2·9	2·7	34	100	2·9	2·7
½	35	105			36	103		
1	35	123			43	126		
2	40	152	5·02	8·54	55	144	4·03	5·82
3	48	182			56	161		
4	55	222			64	190		
5	58	294	9·7	16·3	69	218	6·33	8·5
6	62	369			71	248		
7	84	460			73	279		
8	114	590	23·6 ±0·57	36·4 ±0·76	79	334	10·7 ±0·26	14·4 ±0·30
9	128	690			91	414		

[1]

	2-hour alternate.				6-hour.			
Day	Plants.	Fronds.	Total Area (P.E. ±2·4 %).	Total Dry Weight (P.E. ±2·1 %). mgm.	Plants.	Fronds.	Total Area (P.E. ±2·4 %).	Total Dry Weight (P.E. ±2·1 %). mgm.
0	34	100	2·9	2·7	34	100	2·9	2·7
½	34	110			34	105		
1	38	121			36	118		
2	43	134	4·03	5·6	45	121	3·6	3·84
3	52	155			45	130		
4	57	179			49	136		
5	62	197	5·9	7·7	52	161	4·67	6·11
6	68	242	7·27	9·45	55	177	5·13	6·66
7	78	280			55	190		
8	79	330	10·3 ±0·25	13·3 ±0·28	55	199	5·97 ±0·14	7·65 ±0·16
9	82	368			58	208		

[1]

TABLE II (350 foot Candles).

Expt. 1. Frond Number.

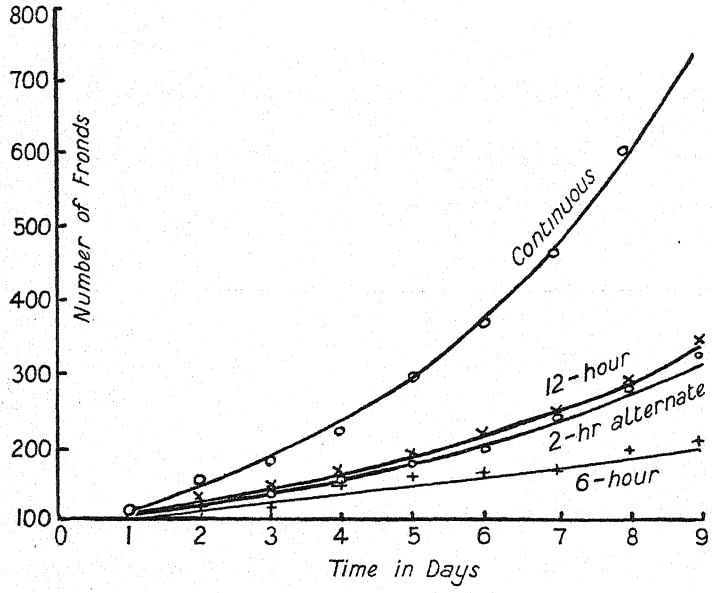
	Continuous. $x = 93{\cdot}5\, e^{0{\cdot}231\, t}$		12-hour. $x = 100\, e^{0{\cdot}127\, t}$		2-hour alt. $x = 91\, e^{0{\cdot}130\, t}$		6-hour. $x = 11{\cdot}42\, t + 91{\cdot}5$.	
	Exp.	Cal.	Exp.	Cal.	Exp.	Cal.	Exp.	Cal.
1.	123	115	103	113	110	104	105	104
2.	152	149	126	128	121	119	118	114
3.	182	185	144	146	134	135	121	126
4.	222	243	161	165	155	155	130	137
5.	294	296	190	190	179	178	136	148
6.	369	374	218	214	197	203	161	160
7.	460	473	248	244	242	234	177	172
8.	590	594	279	277	280	267	190	184
9.	—	—	334	313	330	308	199	194

[1] Relay failed.

growth has been hindered by a factor which is presumably deficiency of light. This question will be discussed in the light of subsequent experiments.

In the course of the experiment the weight per unit frond rose in the 'continuous' colony, and remained about the same in the other colonies.

In this experiment the fronds were counted at 10 a.m. and 4 p.m., for it was thought that some light might be thrown upon the question of diurnal



TEXT-FIG. 4. Experiment 1. Intensity: 350 foot candles. Curves of closest fit; frond number plotted against time.

rhythm in division. The figures, however, gave no indication of any such rhythm, and they have not been included.

6. EXPERIMENT 2.

Light: 700 foot candles, 100 watt lamps; begun June, 1928.

Under an intensity of 700 foot candles growth was much more rapid. The plants had been under this light intensity for five days before the beginning of the experiment with a 14-hour light period, and the fronds were considerably bigger than those under an intensity of 350 foot candles.

The colonies under continuous, 12-hour, and 2-hour alternate periods, increased exponentially. The closeness of agreement between the '12-hour' and the '2-hour alternate colonies' is again very striking. It will be noticed from Table IV that the '2-hour alternate' colony shows a clearly marked

TABLE III (700 foot Candles).

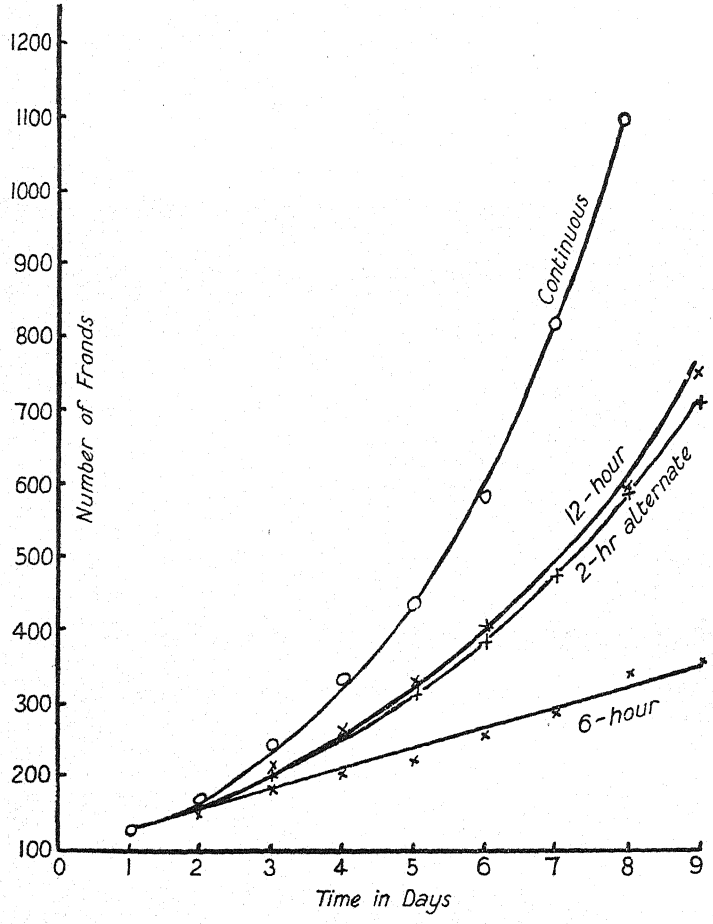
	Continuous.				12-hour.			
Day	Plants.	Fronds.	Total Area (P.E. ±2·4 %).	Total Dry Weight ±2·1 %.	Plants.	Fronds.	Total Area (P.E. ±2·4 %).	Total Dry Weight ±2·1 %.
0	22	100	5·55	12·4	22	100	5·55	12·4
1	23	125			29	127	7·23	15·7
2	26	169	9·48	21·1	31	166		
3	30	246			63	213	11·81	19·8
4	43	336	19·39	42·1	71	258		
5	62	436			92	328	19·05	30·8
6	84	586	37·1	77·0	104	402		
7	109	817			124	473	27·5	41·6
8	164	1090	76·2 ±1·84	138·5 ±2·90	127	579		
9	231	1500			155	737	42·0 ±1·0	63·8 ±1·34
10	305	1950			186	899		

	2-hour alternate.				6-hour.			
Day	Plants.	Fronds.	Total Area (P.E. ±2·4 %).	Total Dry Weight ±2·1 %.	Plants.	Fronds.	Total Area (P.E. ±2·4 %.)	Total Dry Weight ±2·1 %.
0	22	100	5·55	12·4	22	100	5·55	12·4
1	27	128			44	120	6·42	12·73
2	42	169	9·27	16·9	53	156		
3	46	208			59	181	9·67	15·3
4	69	267	5·18	25·9	89	204		
5	84	316			95	220	12·5	18·9
6	94	392	22·85	38·2	95	256		
7	107	475			104	288	15·8	22·5
8	131	594	34·9 ±0·8	59·0 ±1·24	108	340		
9	152	718			115	367	19·0 ±0·45	25·3 ±0·53
10	187	870			129	418		

TABLE IV (700 foot Candles).

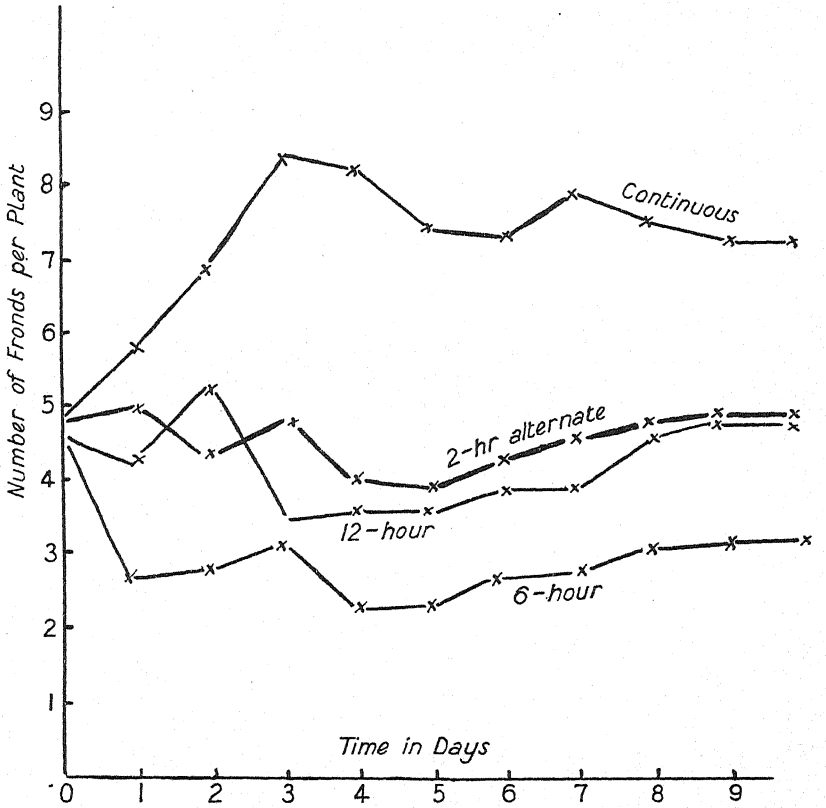
	Expt. 2. Frond Number.							
	Continuous. $x = 90{\cdot}5\, e^{0{\cdot}316\, t}$		12-hour. $x = 106\, e^{0{\cdot}220\, t}$		2-hour alt. $x = 115\, e^{0{\cdot}203\, t}$		6-hour. $x = 29{\cdot}4\, t + 89{\cdot}0$	
	Exp.	Cal.	Exp.	Cal.	Exp.	Cal.	Exp.	Cal.
1.	125	124	127	132	128	135	118	120
2.	169	167	166	164	169	168	148	156
3.	246	235	213	205	208	208	177	181
4.	336	320	258	256	267	253	207	204
5.	436	438	328	322	316	315	236	220
6.	586	634	402	401	392	387	266	256
7.	817	818	473	498	475	476	295	288
8.	1090	1095	580	615	594	588	324	340

rhythm. This rhythm is evident to a lesser degree in the 2-hour alternate colony at 350 foot candles (see Text-fig. 4). In Table IV are set out the experimental and the calculated values for frond number.



TEXT-FIG. 5. Experiment 2. Intensity: 700 foot candles. Curves of closest fit; frond number against time.

The relationship between external conditions and weight per unit frond will be discussed later. It will be seen from Text-fig. 6 that there is a most striking adjustment to external conditions of the number of fronds per plant in the course of the experiment. Experience has shown that darkness facilitates the splitting up of the plant into separate fronds, and all the experiments exhibit a relatively smaller number of fronds per plant at the 12-hour and 6-hour periods than at the continuous period.



TEXT-FIG. 6. Experiment 2. Number of fronds per unit plant.

7. EXPERIMENT 2 *a*.

Light: 700 foot candles, 100 watt lamps; begun 1 September, 1928.

Immediately Experiment 1 was finished the illumination was increased to 700 foot candles, and the fronds in the continuous and 12-hour colonies counted for five days. The object of the experiment was to ascertain whether Experiment 2, performed in June, would give the same results when performed in September, i. e. whether there is any seasonal change in the rate of growth of the colonies. The results are set out in Table V.

TABLE V (700 foot Candles).

	Expt. 2*a*. Continuous.		Frond Number. 12-hour.	
	June	Sept.	June	Sept.
1.	100	100	100	100
2.	125	145	127	120
3.	169	184	166	144
4.	246	234	213	190
5.	336	331	258	230

The agreement, together with the evidence of the control experiment in Experiment 3 (see p. 345), is sufficient to show that the differences in growth in June (Expt. 2) and September (Expt. 1) are not due to the fact that the experiments were conducted at different times of the year.

8. Experiment 3.

Light: 1,400 foot candles, 200 watt lamps; begun 20 November, 1928, with 'control' experiment at 320 foot candles; continuous illumination.

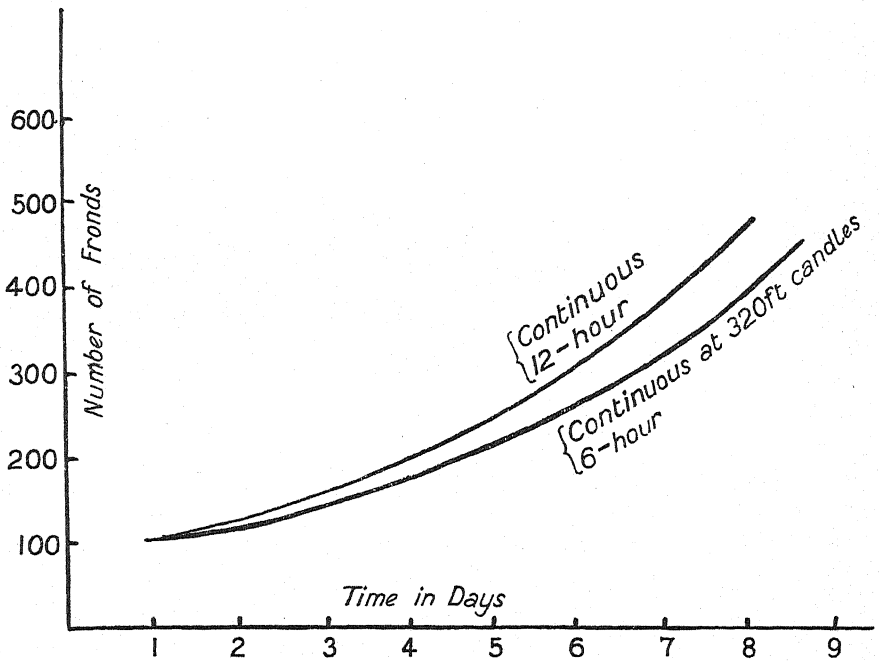
Before the beginning of the experiment the plants were in another growth chamber in continuous light at an intensity of about 650 foot candles. On the third day the pH changed to 5·0; but the solution was immediately replaced.

Table VI (1400 foot Candles).

	Continuous.			12-hour.		
Day	Fronds.	Total Area (P.E. ±2·4 %).	Total Dry Weight ±2·1 %.	Fronds.	Total Area (P.E. ±2·4 %).	Total Dry Weight (P.E. ±2·1 %).
0	100	3·24	11·1	100	3·24	11·1
1	112			107		
2	126	3·77	12·4	144	4·59	(15·8)
3	156			166		
4	205	4·98	18·9	198	5·33	16·2
5	235			241		
6	330			323		
7	394	9·57	32·1 ±0·67	396	8·80	22·0 ±0·46
8	482	11·7 ±0·28		471	10·5 ±0·25	

	6-hour.			Continuous at 320 foot Candles.		
Day	Fronds.	Total Area (P.E. ±2·4 %).	Total Dry Weight (P.E. ±2·1 %).	Fronds.	Total Area (P.E. ±2·4 %).	Total Dry Weight (P.E. ±2·1 %).
0	100	3·24	11·1	100	3·24	11·1
1	108			108		
2	130			135		
3	151	4·52	12·05	154	4·48	13·32
4	198			171		
5	224			200		
6	282	7·14	13·85	264	6·82	15·8
7	335			338		
8	360	8·25 ±0·20	14·5 ±0·30	395	9·0 ±0·21	19·62 ±0·41

The values for increase in frond number, area and dry weight, for all the light periods, lie conformably upon exponential curves. Growth was



TEXT-FIG. 7. Experiment 3. Intensity: 1,400 foot candles and continuous at 320 foot candles. Curves of closest fit; frond number against time. Only two are drawn, since the other two overlap these. The experimental values cannot be included on the graph, because the curves lie too close together. The 'closeness of fit' can be estimated from Table VII.

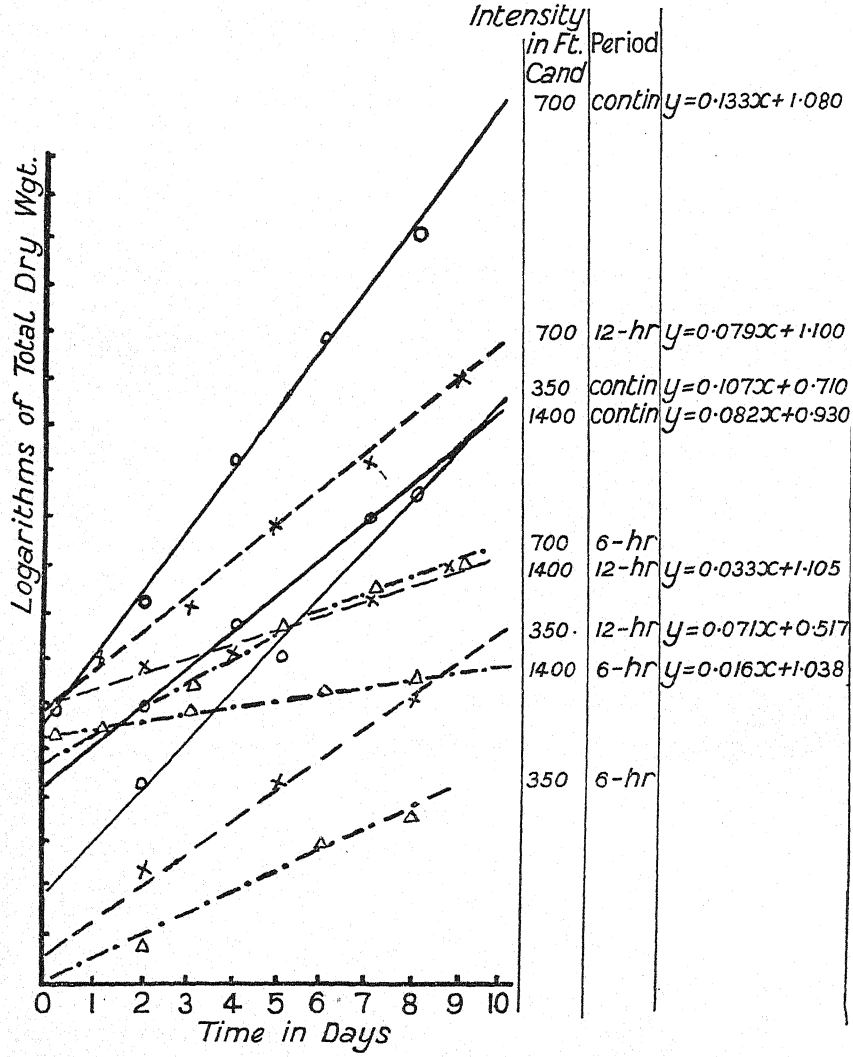
markedly less than at 700 foot candles. It is clear that, under the conditions of the experiment, the optimum light intensity has been exceeded. The results are represented graphically in Text-figs. 7 and 8. The closeness of fit can be seen from Table VII.

TABLE VII.

Expt. 3. Frond Number.

	1,400 foot Candles.				320 foot Candles.			
	Continuous. $x = 84{\cdot}3\, e^{0{\cdot}218\, t}$		12-hour. $x = 87{\cdot}7\, e^{0{\cdot}209\, t}$		6-hour. $x = 88{\cdot}7\, e^{0{\cdot}1905\, t}$		Continuous. $x = 84{\cdot}0\, e^{0{\cdot}194\, t}$	
	Exp.	Cal.	Exp.	Cal.	Exp.	Cal.	Exp.	Cal.
1.	112	104	107	108	108	107	108	104
2.	126	128	144	133	130	130	135	125
3.	156	160	166	165	151	157	154	151
4.	205	199	198	203	198	190	171	182
5.	235	247	241	251	224	229	200	221
6.	330	308	323	308	282	277	264	267
7.	394	391	396	380	335	334	338	324
8.	482	473	471	466	360	404	395	394

Since these colonies were growing under supra-optimal conditions, it was thought that the curves of increase might more closely follow a relation



TEXT-FIG. 8. Experiments 1, 2, and 3. Curves of closest fit; logarithms of dry weight × time.

$\frac{dx}{dt} = \frac{Kr}{t^n}$, where K is the original value of x, and where n is greater than 0 and less than 1. This was the equation which Gregory fitted to his supra-optimal temperature curves (5). But attempts to fit the values from Experiment 3 to such 'damped' exponential curves were not successful. The values agree more closely with an ordinary exponential curve of the

form $x = Ce^{rt}$. It will be noted that even the 6-hour colony gave values falling upon an exponential curve.

At this light intensity, namely, 1,400 foot candles, the colony at the continuous period rapidly began to show abnormal features; these also appeared to a smaller degree in the colony at the 12-hour period. The plants developed a great many overlapping fronds which had to be separated from one another for counting. The colour of the fronds changed from deep green to a pale yellowish green, and they became speckled with white sunken areas.[1] The several fronds of the same plant, instead of lying in the plane of the solution, became tilted up against one another so that the light fell upon them obliquely. The fronds were thicker at this light intensity than at any other intensity employed. Within the duration of the experiment the initial relative growth rate was maintained. Growth at 320 foot candles does not differ very significantly from growth at 350 foot candles (Expt. 1).

9. Errors.

The possible errors to which the experimental data are open can be classified under five heads:

1. Want of uniformity in the experimental colonies.
2. Inequalities of conditions in the four growth chambers.
3. Errors involved in sampling.
4. Errors due to rise in temperature of the plants as they absorb light.
5. Errors due to any seasonal effect on the growth of the colonies, whereby experiments performed at different times of the year would not give similar results, nor be strictly comparable with one another.

1. To find the magnitude of the first group of errors, four colonies, taken at random from a uniform population, were grown for five days under exactly the same conditions in the four growth chambers, and were counted every day. It was found that on the fifth day the probable error of the number of fronds in any one colony did not exceed 1·07 per cent. From this value, the approximate probable error of the index r in the exponential equation representing growth under these conditions can be calculated. For let

$$100 = e^{r_1 t} \text{ and } 101{\cdot}07 = e^{r_2 t}, \text{ then:}$$

$$1{\cdot}01 = e^{(r_2 - r_1)t}, \text{ i.e. when } t = 1,\ 1{\cdot}01 = e^{(r_2 - r_1)},$$

whence $(r_2 - r_1) = 0{\cdot}01$, for the growth rate at 700 foot candles (the intensity at which this test was carried out). That is, a difference greater than 0·03 in the value of r can be regarded as significant.

2. A second source of error, included in the value for the probable error calculated above, lies in the inequalities of light intensity, degree of

[1] In a subsequent experiment, it was noticed that after three days, fronds under continuous illumination at 1400 f. c. corresponded in colour with Chrysolite Green, xxxiii. 27 b., and fronds at 700 f. c. to Scheele's Green, vi. 33. i. of Ridgeway's colour standards (6).

aeration, &c., in the four growth chambers. It was impossible to read the light intensity more accurately than to within 50 foot candles, especially at higher intensities. The effect of these inequalities was minimized by changing the colonies from one chamber to the next every day.

3. The errors involved in sampling have already been considered (1). From time to time determinations of the sampling error were repeated.

4. A fourth error might have arisen due to a rise in temperature of the fronds as a result of absorption of radiant energy. The rise in temperature, however, was found to be very small, and any error due to it would be insignificant. This was ascertained by threading one junction of a thermo-electric couple under the epidermis of a *Lemna* plant, while the other junction was in the solution. The rise in temperature under a cooled light of 2,000 foot candles intensity was found to be less than 0·4° C.

5. Finally, it was necessary to ascertain whether there is any seasonal effect on growth, i. e. whether the same experiment performed at different times of the year would give the same result. The values for these comparisons have already been given (p. 343). In no instance have significant differences been found.

10. Discussion.

General Efficiency. In the series of experiments described, since light is the only varying factor, the relative efficiency of light in relation to growth can be expressed by $\frac{1}{x} \times \frac{dx}{dt} \times \frac{1}{L}$, where x = frond number, area, or dry weight; t = time; and L = the relative amount of light received. Where the curves of growth have been fitted to equations of the exponential type, the relative efficiencies can be found by substituting the proper values in the right-hand side of the equation:

$$\frac{1}{x} \times \frac{dx}{dt} = r,$$

and dividing by the relative amount of light received. Let $L = 1$ for a 6-hour period at 350 foot candles; then $L = 2$ for a 12-hour period at 350 foot candles; $L = 4$ for a 12-hour period at 700 foot candles, and so on. Since the results are relative, t can be made unity and the following equation holds:

$$\text{Efficiency } (E) = \frac{dx}{dt} \times \frac{1}{x} \times \frac{1}{L} = \frac{r}{L}.$$

From this equation, Table VIII has been calculated. It reveals the striking fact that the relative efficiencies of increase in frond number, and increase in dry weight, differ markedly under the same conditions. Both for frond number and for dry weight the highest efficiencies tend to be at 350 foot candles. The efficiency for increase in frond number does not drop significantly until the 12-hour period at 1,400 foot candles is reached; whereas the efficiency for dry weight increase

has dropped 40 per cent. in the colony under 700 foot candles and a 12-hour period. That absorption of light increases with increasing light intensity is confirmed by the fact that the efficiency of frond increase is maintained up to the 6-hour period at 1,400 foot candles. The reduction of efficiency at 1,400 foot candles (12-hour, and continuous periods) may be due to reduced absorption since, as described on p. 346, the fronds under these conditions were much paler in colour than the normal fronds. The pallor is due to paucity or disintegration of the chlorophyll, a condition which would be expected to result in a reduced absorption of light.

TABLE VIII.

Intensity (Foot Candles).	Duration. Hours.	L.	Frond Number. r	Frond Number. E.	Dry Weight. r	Dry Weight. E.
1,400	continuous	16	0·218	0·014	0·189	0·012
1,400	12-hour	8	0·209	0·029	0·076	0·009
1,400	6-hour	4	0·194	0·048	0·037	0·009
700	continuous	8	0·316	0·039	0·306	0·034
700	12-hour	4	0·220	0·055	0·182	0·046
700	6-hour	2	—	—	—	—
350	continuous	4	0·231	0·058	0·247	0·062
350	12-hour	2	0·127	0·064	0·163	0·082
350	6-hour	1	—	—	—	—

The probable error of any value of the efficiency is about 5 %.

In the dry-weight series the diminution of the efficiency is evident at the 12-hour period of 700 foot candles. This cannot be due to a reduced percentage absorption of light unless the reduction were specific to the rays causing assimilation, which is inherently improbable. Nor can it be attributed to the accumulation of the products of photosynthesis, for the amount of light received by the colonies at the 6-hour period of 1,400 foot candles and the 12-hour period at 700 foot candles are equal, and the former has a longer dark period in which to reduce any accumulated products.

Two suggestions may be advanced to account for the reduction in efficiency of the dry-weight increase:

1. That although the energy is absorbed the formation of material is limited by some internal factor the action of which is not accelerated by increase of light intensity, e. g. the 'dark' reaction of photosynthesis.

2. That there is a harmful influence of the supra-optimal light intensity which affects the assimilatory process more profoundly than the process of frond formation.

This second hypothesis is supported by the fact that not only is the efficiency lower, but the rate of growth itself is smaller at 1,400 foot candles; the pallor and the oblique orientation of the fronds already described also favour this view.

The fact, however, that the dry-weight efficiency is reduced even at 700 foot candles suggests that both the above hypotheses may apply.

The Interrelationship of Duration and Intensity of Light. This interrelationship is best illustrated by the three-dimensional model (Pl. VII). At low intensities, light added as 'duration' or intensity, *a–b* or *a–c*, has the same effect upon growth. The contours *a–c–p*, *b–d–g*, *a–b*, *c–d*, so far as the evidence of the experiments goes, approximate to straight lines. The optimum for light probably occurs between 700 and 1,400 foot candles. Further experiments are being carried out to determine the point more closely. If the amount of light given during the 12-hour period at 700 foot candles is multiplied by two, either by doubling the duration, *d–g*, or by doubling the intensity, *d–e*, then in the former case the growth is proportionately increased, and in the latter case it is very much reduced. The contour at 1,400 foot candles, *k–e–m*, resembles a typical curve in which one factor has assumed control, i. e. has become almost limiting.

The most striking feature of the model is the diminution of growth rate at intensities of light somewhat above 700 foot candles. It might have been expected that the growth rate would rise to a maximum value with increasing light intensity, and would remain at this value for supra-optimal light intensities until the intensities became very high. Clearly, however, the light has some harmful influence at 1,400 foot candles. It is significant that the relative 'depression' of growth is greatest under continuous light.

That the depression is not due to some extraneous factor, such as poison, is shown by the fact that the 'control' colony at 320 foot candles, in the same solution as the colonies at 1,400 foot candles, gave results in agreement with expectation, and these fit conformably to the curves between 0 and 350 foot candles.

Similar models constructed for total dry weight and total area after eight days show essentially similar results.[1]

It may be suggested tentatively that the depression of growth processes at a light intensity of 1,400 foot candles is due to some harmful effect of the light intensity upon the chlorophyll mechanism.

The Constant of Frond Weight. If the formation of fronds were merely the redistribution of material according to the amount of 'income', the number of fronds formed per unit 'capital' of dry weight should be in every instance the same; this, however, is not the case. In most experiments the value $\frac{\text{dry wt.}}{\text{fronds}}$, calculated from day to day, was found to settle down after a few days to a constant level, *different for different sets of*

[1] This seems to corroborate the finding of Gregory (7) that leaf area is proportional to total radiation, except under supra-optimal conditions. That this relationship holds has lately been disputed by Davis and Hoagland (8).

conditions. This value will be known as the *constant of frond-weight*, since it measures the amount of frond formation per unit capital of dry weight.

The values for the constant of frond weight after eight days were:

TABLE IX.

$$\text{Const. of Frond Weight } (C) = \frac{\text{Number of Fronds.}}{\text{Dry Weight.}}$$

Intensity Foot Candles.	Duration.	Const. of Frond Weight.	State of C after 8 days.
1,400	continuous	12	steady
1,400	12-hour	24	rising
1,400	6-hour	30	rising
700	continuous	8	steady
700	12-hour	11	steady
700	6-hour	14	slightly rising
350	continuous	18	steady
350	12-hour	25	steady
350	6-hour	26	steady
320	continuous	19	slightly rising

The fact that the colonies require about a week to reach a constant value for C does not necessarily indicate that the value is changing in the colony. It is explained by the fact that the original frond population is still present in the colony, and is probably not affected so profoundly by a change of conditions as are new fronds actually formed under those conditions. The influence of these original plants upon the gross weight per unit frond of the population will only gradually be diminished as the new fronds more and more outnumber the old. As would be expected, if this hypothesis is correct, the curves of C against time closely resemble exponential ones.

There are striking differences in the number of fronds formed per unit of dry weight. It is noteworthy that at each light intensity the highest constants of frond weight occur at the 6-hour light period, and the lowest at the continuous period. This would indicate some correlation between frond formation and the length of the dark period. The high and rapidly rising constants under 1,400 foot candles at the 12-hour and 5-hour periods indicate that this light intensity is inhibiting assimilation without having an equally serious inhibition upon frond formation. Similarly at 350 foot candles the food resources of the plants are low, but differentiation continues unchecked, resulting in high values for the constant. At 700 foot candles the conditions are about optimum for assimilation, and there may be a surplus of food available, which would result in a low value for the constant of frond weight.

Reference to Tables I and III will show that the colonies under 700 foot candles and a 6-hour period, and 350 foot candles and a 12-hour period were receiving the same amount of light, and that their increase, within the

duration of the experiment, was approximately the same. But the values at the former light intensity lay rhythmically about a straight line, while those at 350 foot candles increased exponentially. In this connexion it is perhaps significant that the constant of frond weight at 700 foot candles is 14, and that at 350 foot candles is 26. That is, 81 per cent. more material enters into the composition of a frond at 700 foot candles and a 6-hour period than at 350 foot candles and a 12-hour period. If, therefore, there were any 'shortage of income' under these light conditions, the following relationship would hold: that the lower the frond weight constant is, and the longer the dark period, the more severely would the unrestricted exponential increase of frond number be 'damped'. This circumstance might explain the difference between the two curves under discussion.

Thickness of the Fronds. It is well known that the thickness of the assimilatory tissue of a leaf is influenced by the intensity of the incident light. The weight per unit area of the fronds (Table X) is some indication of their thickness; and the influence of light intensity is illustrated by the following: At 350 foot candles, fronds were of about the same thickness for all durations of light, and the value of weight per unit area was relatively low. At 700 foot candles the general level of thickness was slightly higher. At 1,400 foot candles the fronds were thicker still, especially those under continuous illumination. This accords with the structure of the typical sun leaf. Even continuous illumination at 1,400 foot candles does not seem above the optimum for such thickening. This thick frond, together with the oblique orientation to the incident light, mentioned on p. 346, constitute two of the characteristics of 'sun leaves'.

TABLE X.

Weight per Unit Area; mg. per cm.^{2}

Intensity Foot Candles.	Periods. Continuous.	12-hour.	6-hour.
1,400	3·6	2·5	1·8
700	1·9	1·5	1·3
350	1·6	1·3	1·2

11. SUMMARY AND RESULTS.

An apparatus is described by which four colonies of *Lemna minor* can be grown under constant conditions of nutrition, temperature, and light intensity. There is also described an arrangement whereby the four colonies can be exposed to four different daily periods of illumination, known as the continuous, 12-hour, 2-hour alternate, and 6-hour periods.

A technique is outlined for the investigation, under these four different light periods, of increase in plant number, frond number, area, and dry weight.

The data are given for experiments at three different light intensities: 1,400, 700, and 350 foot candles.

The errors involved in the method are discussed.

The curves of increase in frond number, area, and dry weight, plotted against time, approximate closely to the exponential type. Equations of the form $x = Ce^r$ have been fitted to the experimental data; with the exception of two sets of data (the 6-hour period at 700 and that at 300 foot candles), whose values lay rhythmically about a straight line. It was found that at light intensities of 700 and 350 foot candles, and for the period of the experiments, growth under alternating periods of light and darkness did not differ significantly from growth under a 12-hour period.

Under the conditions of the experiments, growth under continuous light is in every instance greater than growth under shorter light periods. Growth increases with increase of intensity of light up to 700 foot candles, but falls markedly at 1,400 foot candles, at which light intensity a harmful effect of the light is evident. The optimum light intensity for the conditions of the experiments lies probably between 700 and 1,400 foot candles.

It is suggested that up to the optimum light intensity the product-law is followed with respect to increments of light. The diminution in rate of growth at supra-optimal light intensities is partly due to a reduction of the chlorophyll; though some additional factor probably operates, since at the shorter light periods a reduction in growth takes place, with apparently no corresponding reduction in chlorophyll content.

The significance of the constant of frond weight, i. e. the number of fronds formed per unit capital of dry weight, is discussed.

From an inspection of the relative efficiencies, expressed as $\frac{r}{L}$, of rate of increase of frond number and dry weight, the differential effect of high light intensity on frond formation and on assimilation can be observed.

Morphological observations, and the relative thickness of fronds grown under different conditions, suggest that under high light intensities, structures similar to those of a typical 'sun leaf' are produced.

I wish to record my thanks to Professor V. H. Blackman for his stimulating interest and helpful criticism.

I have pleasure also in thanking Mr. Thet Su, who built the greater part of the apparatus described on page 334, and who obtained most of the data in the second experiment; and Mr. W. Shaw, who designed the time-switch described on page 335.

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EXPLANATION OF PLATE VII.

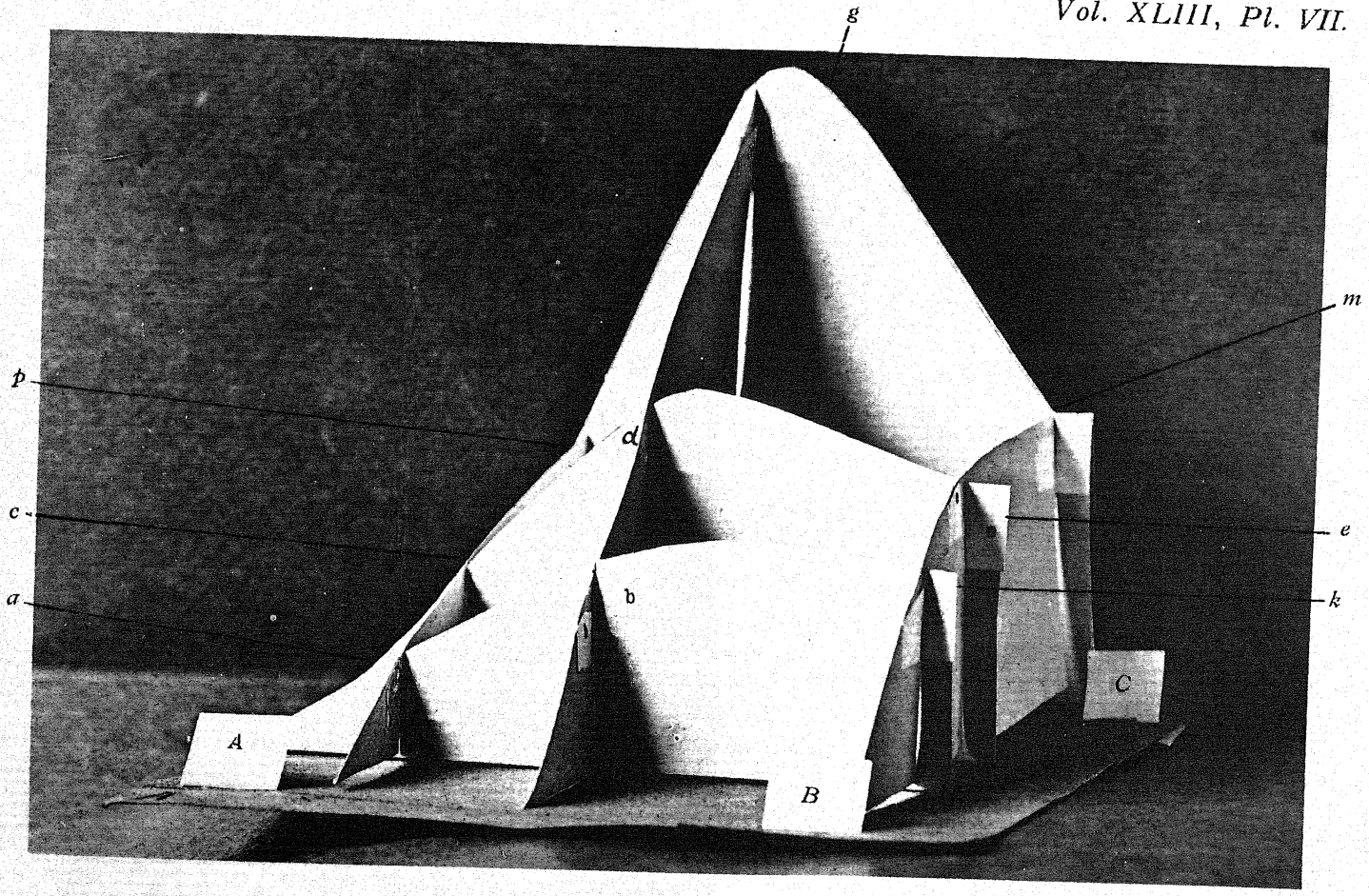
Illustrating Mr. Ashby's paper on the Interaction of Factors in the Growth of *Lemna*.

The figure is a photograph of a cardboard model illustrating the interaction of intensity and duration of light upon frond number.

The ordinates represent the number of fronds in the several colonies after eight days growth.

The abscissae A–B represent increments of light as *intensity*; the abscissae B–C represent increments of light as *duration*.

a =	350	foot candles,	6-hour duration.
c =	350	,,	,,	12-hour duration.
p =	350	,,	,,	continuous.
b =	700	,,	,,	6-hour duration.
d =	700	,,	,,	12-hour duration.
g =	700	,,	,,	continuous.
k =	1400	,,	,,	6-hour duration.
e =	1400	,,	,,	12-hour duration.
m =	1400	,,	,,	continuous.



ASHBY — GROWTH OF LEMNA.

Huth, London.

On a Variety of Scolopendrium vulgare [1] that bears Sporangia on the Prothallus.

BY

WILLIAM H. LANG, F.R.S.

(*Barker Professor of Cryptogamic Botany in the University of Manchester.*)

With Plate VIII and six Figures in the Text.

INTRODUCTORY.

IN 1896 and 1898 the production of sporangia on Fern prothalli was recorded and described as occurring in cultures of *Scolopendrium vulgare*,[1] var. *ramulosissimum*, and *Nephrodium dilatatum*,[1] var. *cristatum gracile* (3, 4). In both these cases it was associated with apogamy, but normal embryos were also borne on prothalli in the cultures. Since embryos were freely produced under normal conditions of cultivation in which the prothalli were watered from above, the presumption was that the deviations from normal development were induced by unknown conditions of cultivation, acting on susceptible varieties of Ferns, but no clear analysis of the causal conditions was obtained. It seemed possible that strong illumination might have played a part, but the prevention of fertilization by avoiding watering from above was probably a more significant change from normal conditions.

No further cases of the production of sporangia on prothalli have since been noticed in numerous cultures that have been under my observation, until the one that is the subject of this paper was met with. There has also been, so far as I know, no clear confirmation of this phenomenon by other investigators. The only exception is a somewhat doubtful example in an undetermined prothallus of Osmundaceous type recorded by Miss L. Pace (5). While her interesting description is strongly suggestive of this interpretation being correct, it involves too many uncertain points to be a critical record.

It therefore appears desirable to give an account of another varietal form of *Scolopendrium vulgare*, which has repeatedly shown the production

[1] Strictly speaking, these names should be given as *Phyllitis scolopendrium*, (L.) Nasm. and *Dryopteris spinulosa*, (Mull.) O. Ktze, but more uniformity and precision is obtained by continuing to employ the names used in the earlier literature of this subject.

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of sporangia and of apogamous buds on some of the large prothalli in old cultures, in which normal fertilization had been prevented by avoiding watering from above.

The parent plant appeared in the Moss-house at Fallowfield a good many years ago and, since no similar form was in cultivation, was most naturally attributed to a stray spore. Its source is therefore unknown. It seems highly improbable that it was a mutant from the normal plants of *Scolopendrium* then growing in the Moss-house. The application of a varietal name to it is of little value since, like such merely descriptive naming of varietal forms of Ferns, it would not indicate a strain with a known history. From the appearance of the fronds it would be termed var. *ramo-digitatum*; in well-grown fronds it shows sori on the upper surface of the frond as well as below. It reproduces truly from sowings of the spores.

Attention was directed to the peculiarities in the constitution of this plant of *Scolopendrium vulgare* in 1922, when a group of sporangia was noticed on a stray prothallus in a culture of the spores of another Fern. These stray prothalli were proved to belong to the plant referred to in the preceding paragraph in two ways. The plants reared from them agreed with the presumable parent Fern, and cultures made from the spores of the suspected plant produced groups of sporangia on some of their old prothalli. Since then, numerous cultures have been made, and the property of occasionally producing sporangia on old prothalli has been found to hold, not merely for cultures of spores of the original plant, but for cultures from the spores of the plants bred from these.

It is thus clear that we are dealing with a varietal form or mutant of *Scolopendrium vulgare,* which breeds true, and is characterized not merely by the ramo-digitate shape of its fronds and by being supra-soriferous, but by peculiarities in its developmental potentialities. As will be shown below, its prothalli produce embryos and young plants in the normal way when cultures of suitable age are watered from above. Even such moderate-sized prothalli have archegonia on the upper side as well as below. This appears to be a very constant feature, and becomes very marked in unfertilized prothalli which attain a considerable size. It is only occasional individuals among these large prothalli which produce groups of sporangia and develop young plants apogamously.

It was hoped that some insight into the conditions determining the development of sporangia on these prothalli might be obtained. But owing to the sporadic way in which the peculiar developments of the old prothalli occur, this has not, as yet, been possible. All that can be done at present is therefore to give a description of the prothalli, and to call attention to features that may be significant if a deeper understanding of the process of development is ultimately attained.

External Morphology.

The prothalli as they develop from the spores have the usual form and appearance. After they have reached the stage with a cushion bearing archegonia below, archegonia begin to be apparent on the upper surface also. This is a quite regular feature of these prothalli. The young plants developed in cultures of this age are attached by a foot in the usual position (Pl. VIII, Fig. 1), and there is no reason to doubt that they are the result of normal fertilization of egg-cells. This process has not been directly observed, but circumstantial evidence is provided by experiments made with a number of cultures of the same age, some of which were freely exposed and watered from above, while others were protected from any access of water from above by glass plates. In the former case a crowded crop of young sporophytes resulted, while only an occasional young plant, or none, appeared in the protected cultures.

In the covered cultures the individual prothalli continue their growth and attain a large size and sometimes considerable thickness; archegonia are abundant on the dorsal surface as well as below. These prothalli may branch dichotomously from the apex and often exhibit profuse gametophytic budding from the margin. In these ways those that persist in an old culture give rise to other individual prothalli. Many of the prothalli in an old culture are thus very irregular and complicated in form.

This irregular and crowded growth prevents the minute examination of individual prothalli from above without disturbing the culture. For this and other reasons it has proved difficult to detect early stages of the further abnormal developments, which, when they occur, only affect a small proportion of the prothalli in any culture. Usually attention is directed to a prothallus by noticing a group of sporangia on the upper surface or apogamously developed buds at the anterior margin, and the initial stages are then of course long past.

From the relations of the parts it is, however, clear that a regular course of development and, resulting from this, a fairly definite morphology, is to be recognized in the prothalli that have produced sporangia and apogamous buds. The relative positions of the various structures hold generally, though they may be obscured in the case of some of the irregularly grown prothalli and modifications are also occasionally met with. The usual condition is illustrated by the prothalli shown in Text-figs. 1 and 2.

The prothallus seen from above in Text-fig. 1 is unbranched, with the usual incised margin and some gametophytic budding, and bears a number of archegonia on the upper surface. Some distance behind the anterior end a group of sporangia (accompanied in this case by a small prothallial growth) is borne. The region in front of the sporangia, leading to the

anterior margin of the prothallus, is relatively thin. From this margin, at one side of the broadened apical depression, a slender, cylindrical, stalk-like structure has grown out: this bears an archegonium and widens out into an apogamous bud, one leaf of which has expanded. Two other features not shown in the figure must be mentioned. The sporangia here, as in most cases, were present not only on the upper surface but on the lower surface immediately opposite. Further, a brown discoloration was dimly visible



TEXT FIG. 1. Prothallus with sporangial group and, at the anterior margin, a bud terminating a stalk-like outgrowth. × 8.

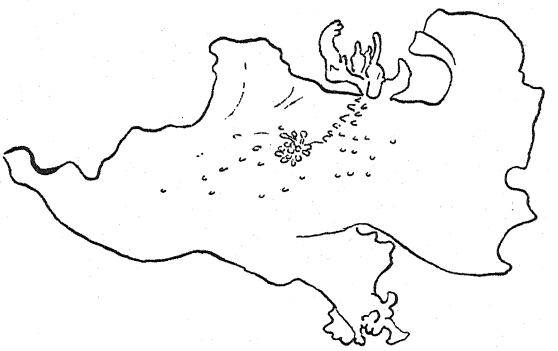
within the bulge just behind the sporangial group. These two points will come out clearly in the account of other specimens, and especially when sections are considered.

The same relative positions of the sporangial group and the apogamous buds are seen in the prothallus represented in Text-fig. 2. In this, which is viewed obliquely from above, the thin lateral portions were bent down so that the structures along the median line were clearly exposed. The thick middle region bears numerous archegonia on the upper side, and these occur not only on the region behind the group of sporangia but between this and the anterior apical margin. From the latter a group of outgrowths has developed, several of which are continuing as apogamous buds with the first leaf recognizable.

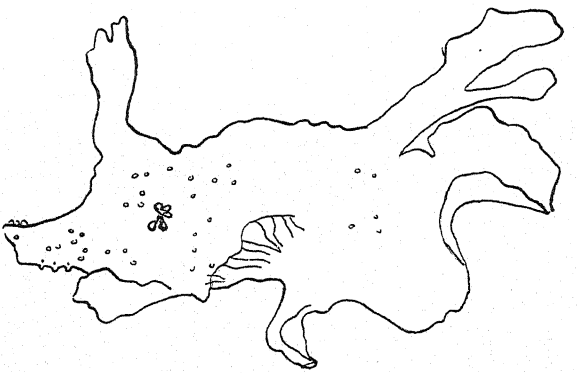
The relation of the sporangial group to the bud or buds in these two examples has been seen in many other prothalli. It naturally suggests that the sporangia were first developed, and at a later stage the buds. This was proved by following the history of some prothalli on which only sporangial groups were present. Thus the specimen shown in Fig. 2, Pl. VIII, was photographed as it grew in a culture and left undisturbed. It gives a very good idea of the appearance of a sporangial group, consisting of numerous whitish sporangia the development of which has been arrested

at a fairly advanced stage. The anterior end of the prothallus is continued as a short, thick, upturned outgrowth, and the tip of this in a few weeks was observed to bear an apogamous bud.

From the evidence afforded by this and other examples, it is reasonable to assume that apogamous buds would have formed later on prothalli that



TEXT-FIG. 2. Prothallus seen obliquely from above, with a sporangial group, and at the anterior margin a number of apogamous buds. × 6.

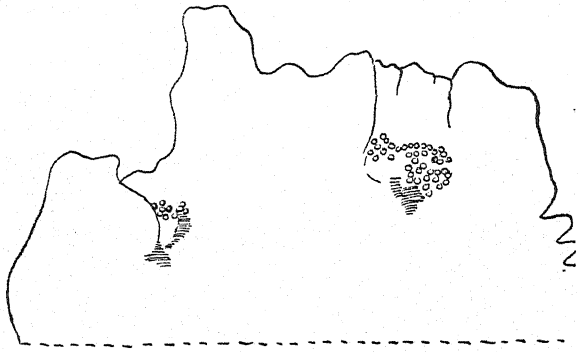


TEXT-FIG, 3. Prothallus of irregular form and thickened anteriorly, with a small group of sporangia on the upper surface. × 8.

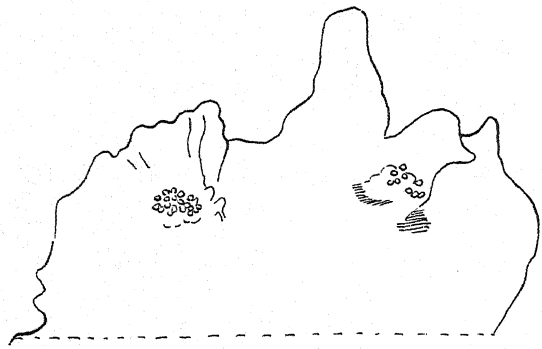
were collected and fixed when only sporangial groups were present. This condition is illustrated by the specimen which is represented from above and below in Text-fig. 4. The prothallus had branched dichotomously. Some distance behind each of the two apical regions a large sporangial group was evident from above. When the prothallus was turned over and viewed from below, a corresponding development of sporangia was found on the other side of each of the swollen regions. A marked brown discoloration in the latter just behind the sporangial groups was very evident in this specimen. The region between the sporangial groups and the corresponding

apical margins was thin relatively to the region where the sporangia were borne, though thicker than the anterior margin in most normal prothalli.

The relative positions of sporangial groups and apogamous buds illustrated by Text-figs. 1 and 2, hold for the great majority of specimens found, and may be regarded as the normal result of the exceptional further



a.



b.

TEXT-FIG. 4. Anterior portion of a dichotomous prothallus seen from above (4 *a*) and below (4 *b*). Behind each apical region is a sporangial group both above and below, and a brown discoloration in the tissue below and behind the sporangial group. × 14.

development of these prothalli. Other relations have, however, been met with in isolated cases, and must be mentioned. Thus in one example in which both sporangia and buds were present, the latter sprang from below the prothallus in the region of the sporangial group, sporangia being mainly developed on the upper surface. In another case an apogamous plant had developed on an outgrowth from the upper surface close to the anterior margin, no recognizable sporangia being present; the outgrowth bore irregular projections, but whether these were best regarded as imperfect sporangia or malformed sexual organs was not ascertained.

Usually when only apogamous buds were visible at the anterior end of a prothallus, careful search, involving the breaking off of buds and prothallial lobes that obscured the region behind the apex, revealed a sporangial group in the ordinary position. This was the case in the prothallus bearing a number of apogamously produced plants that is represented in Text-fig. 5. This specimen was very irregular in form. Its lobes are reflexed and their margins at places thickened in a way that often precedes the growth of



TEXT-FIG. 5. Prothallus of irregular form, bearing at its anterior end a number of apogamously produced young plants. × 6.



TEXT-FIG. 6. Anterior portion dissected out from the specimen in Text-fig. 5, showing the presence of a large group of sporangia and imperfect linear leaves growing from the anterior margin. × 20.

numerous, small, secondary prothalli. No sporangia were visible, but, when the prothallus was broken up, its main apical region was found to have the appearance represented in Text-fig. 6. There was a large sporangial group, and in front of it a marginal outgrowth with a number of cylindrical growths that presented the features of imperfectly differentiated leaves. Cylindrical growths of this kind were also present at the bases of some of the buds borne on this prothallus. Such a passage to a definite shoot through preliminary leaf-like growths, irregularly placed with reference to one another, was not infrequent.

In almost every example examined, the alteration of the prothallus associated with the production of sporangia affected the whole thickness, and sporangia were present above and below. In one case, however, a very small localized group of sporangia was present on the upper surface of a peculiarly grown and much thickened prothallus. The external appearance of the specimen is represented in Text-fig. 3.

It would, of course, be possible to give further examples of the external form of these prothalli, but they would only show differences in detail. Enough have been described to indicate the usual relation of sporangia and buds in the prothalli that undergo apogamous development and to serve as an introduction to the consideration of the structure.

Internal Structure.

The structural features of such prothalli as have been described above have been studied in series of longitudinal sections of a considerable number of examples. Some of the specimens were fixed for the sporangial groups before apogamous buds had originated, and few of the others had the buds placed so as to be instructively shown in connexion by a section passing through the sporangial group. Pl. VIII, Fig. 3, will serve to show the relative positions of sporangia and buds in a longitudinal section. Other sections of the series of this specimen provided the additional information that sporangia were present below as well as above and that a few abnormal sexual organs were associated with the buds. The connexion of the latter with the prothallus is often by a slender stalk not lying in one plane and is therefore better studied by external observation. Since little additional information has so far been obtained from the study of sections of the buds, attention will be mainly directed to the structure of the region bearing the sporangia.

The main features, that repeat with variations in most of the speimens, are illustrated by the longitudinal sections in Pl. VIII, Figs. 4, 5, 6. There is an abrupt transition from the prothallial tissue of the cushion to the altered further growth. The transition is marked by the presence at the transition region of cells of prothallial type, the walls of which have become swollen and brown. The thickness of the prothallus diminishes rather rapidly on passing from the normal prothallial region to the further growth, which by unknown changes has altered its developmental possibilities. The results of the alteration are evident immediately in front of the transition region both in the superficial tissues and internally. The central tissue forms a rather massive vascular strand which occupies the base of the changed growth where this is diminishing in thickness. The sporangia are developed from the superficial layers of tissue both above and below the strand. This sporangium-bearing layer may come immediately outside the sheath of the vascular strand, as is best seen in Pl. VIII, Fig. 5, and holds also for the specimen in Pl. VIII, Fig. 4. The specimen in Fig. 6 shows that there may be several layers of cells outside the vascular strand in this region. Figs. 4 and 5 show crowded groups of sporangia of various ages on the upper and lower sides of this region of the prothallus. In Pl. VIII, Fig. 6, the sporangia occupy the same positions, but are not so crowded, and there are irregular growths of the surface bearing them.

These three examples, to which others only differing in details could be added, show the main structural features of the region bearing the sporangia and its relation to the prothallial tissue behind and the apparently prothalloid continuation in front. In all cases the transition from the prothallial tissue is fairly definite. It is marked by changes in the size and appearance of the cells that are not easy to define, and is often accentuated by the occurrence at the transition region of cells, some or all the walls of which have swollen up and assumed a bright yellow or brown colour, while their protoplasts appear unhealthy or dead. In the median sections in Pl. VIII, Figs. 5 and 6, the brown-walled cells are most evident immediately behind the base of the vascular strand, but when the series were considered it was evident that they frequently extend up the two sides of the strand, forming a cup-like investment of its base. This is the explanation of the distribution of these cells seen in Pl. VIII, Fig. 4, which is a section somewhat to the side of the median plane of the vascular strand. These brown-walled cells account for the appearance of this region which was noted in reference to the external examination of these prothalli (cf. Text-fig. 4). In connexion with them it may be noted that a small group of similarly altered cells is not infrequently present below the venter of an unfertilized archegonium whether on the upper or lower side of these prothalli. The vascular region or strand is widest at its posterior or basal end, and tapers off as it ends in the hinder part of the anterior prothalloid prolongation. It is more or less clearly bounded by a sheath of flattened cells, which may be double and exhibit peculiar deeply staining contents. The tissue enclosed by this sheath may be largely parenchymatous or it may have short, longitudinally placed tracheides distributed through it, or it may form a solid strand of tracheides (cf. Pl. VIII, Fig. 8). The tracheides may be in immediate contact with the sheath, and there is never any appearance to suggest the presence of phloem around or in the vascular strand. Outside of the vascular strand and usually close to it is the external layer of tissue from which the crowded sporangia arise. It is noteworthy that in almost all cases the sporangia are equally developed on the upper and lower sides, the altered region bearing them appearing as the direct continuation forward of the prothallus. The anterior prolongation in front of the sporangial region varies in thickness. When, as is sometimes the case, it is only three cells thick, the upper and lower layers can be traced back to the sporangium-bearing tissue, while the middle or inner layer is continuous with the region of the vascular strand behind. The cells of the anterior prolongation often contrast somewhat, in the fixed and stained condition, with those of the posterior region of the prothallus, but, on the whole, the tissue has a prothalloid appearance. It sometimes bears archegonia, but it would be an unsafe assumption to regard it as being of completely typical prothalloid nature. It is continuous with the tissues of the buds when these are developed, though the connexion may be

a very slender strand of apparently prothalloid tissue, and archegonia and antheridia may occur close to the transition to a bud.

Attempts made to obtain early developmental stages leading to the fairly regular structure of the sporangium-bearing region have not, so far, been successful. The occurrence of prothalli proceeding to these departures from the normal structure is so occasional and sporadic in the cultures, that, when large prothalli have been examined at random, they have proved to be normal. In cases where suspicion of an early stage in the departure from the normal was aroused the changes have, on the other hand, proved to be somewhat advanced. Thus in several prothalli selected as peculiar but not yet bearing evident sporangia, the vascular tissue and anterior prolongation were already present and the earliest sporangia were found in a young condition. Pl. VIII, Fig. 7, shows the change of tissue in such a prothallus on passing forward from the thick prothallial region on the right to the thinner anterior prolongation composed of differently staining cells. A vascular region, with a few cells with brown walls at its base, is present and groups of young sporangia had developed both above and below; the section figured only shows the sporangia on the lower side. The corresponding transition region of another prothallus (Pl. VIII, Fig. 8) shows a few brown-walled cells behind a well-marked vascular strand, and young sporangia above and below this. It seems clear from these examples that the sporangia do not arise until the further growth of the prothallus has taken place and the vascular strand in the base of the changed region has become differentiated.

Other specimens that had no sporangia proved to be, in various ways, equally advanced. They were alternative modes of construction, rather than initial stages leading to the usual type. Thus the specimen shown in longitudinal section in Pl. VIII, Fig. 9, has a transition from the prothallus to the vascular region that does not differ essentially from that seen in Pl. VIII, Fig. 6. There is a rather wide zone of tissue outside the vascular strand, and the outer tissue extends into a number of small projections. The anterior prolongation thinned off gradually in front, the vascular strand dying out in its base in the usual fashion. No sporangia were present on this specimen. Its interesting and exceptional feature was that the tissue of the anterior prolongation had well-marked intercellular spaces. It was thus of more sporophytic character than usual, and more suggestive of an approach towards a rudimentary form of leaf.

Another specimen, which agreed with that last described in bearing no sporangia, but was otherwise of a different type, is illustrated in Pl. VIII, Fig. 10. Here the prothallus underwent a considerable diminution in thickness on passing to the anterior prolongation, but the latter appeared distinctly prothalloid, bearing archegonia both above and below. One of the archegonia above has been transformed into a vegetative outgrowth seen in the

figure. In the position of the sporangium-bearing region of the more usual type there was a swelling, especially prominent towards the upper side. This had within it an irregular development of vascular tissue, the tracheides being embedded in a small-celled tissue. Around, and especially below this, and extending forward in an exceptional fashion, were cells with brown, thickened walls, such as usually occur in the transition region. Prothalloid as the tissue of the anterior prolongation was in this case, an isolated small group of tracheides occurred within it; this was shown by another section of the series.

In all the examples described, even in that last mentioned, the change in the region of the vascular strand or the sporangium-bearing region affected the whole thickness of the prothallus, the further direct growth of which formed the more or less altered anterior prolongation. The only instance of a localized development of sporangia, not affecting the internal structure, was that represented in Text-fig. 3. A longitudinal section passing through the sporangial group on this prothallus is shown in Pl. VIII, Fig. 11. The prothallial nature of the tissue, including that forming the small dorsal bulge, is evident and archegonia are seen above and below. Along the side of the bulge that is directed away from the apex there is a small patch of tissue of different type and the sporangia spring from this. No tracheides are present in this prothallus. There are one or two cells with somewhat swollen brown walls immediately behind the altered tissue, but it is doubtful whether any significance can be attached to them. While it seemed possible that the occurrence of this sporangial group was to be related to the site of an altered dorsal archegonium, no evidence of this was afforded by the series of sections through the small bulge of tissue.

To complete the examples giving an idea of the relations of parts in these abnormal prothalli the structural features of one other specimen may be described. This was of special interest because it showed in the same line of growth (*a*) the prothallial tissue of the cushion, (*b*) a sporangial group with the underlying vascular tissue, (*c*) a prothalloid continuation with archegonia, (*d*) a second sporangial group with underlying vascular tissue, (*e*) a prothalloid prolongation, (*f*) apogamous buds associated with ill-developed sexual organs. These features are not shown in any one longitudinal section of the series, but sufficient evidence for them is afforded by the two sections represented in Pl. VIII, Figs. 12, 13.

That in Pl. VIII, Fig. 13, starts with the normal prothallial tissue on the extreme left and is almost median for the first sporangial group and its vascular strand. Sporangia are present both above and below. The brown-walled cells at the transition zone are well marked. The anterior continuation is at first thin, but becomes thicker and prothalloid; archegonia were here present on both sides. At the thickened anterior end of this growth was the second smaller group of sporangia. Though not cut in the median

plane by this section, its position is indicated by the sporangial capsules above and below. As the other sections of the series showed, it was a repetition on a somewhat smaller scale of the first group, with brown-walled cells at the transition region and a vascular strand with a well-marked sheath. This strand died out at the base of the second prothallial continuation which is seen on the right-hand side of Pl. VIII, Fig. 13.

The section in Pl. VIII, Fig. 12, is selected to show the apogamous buds on the margin of the second prothallial continuation. It misses both sporangial groups. The position of the hinder one is indicated by the capsules above and below the prothallus. No sporangia of the second group are visible, but the approximate position of the group is indicated by crosses. The anterior portion of the prothallial continuation in front of this is seen to have thickened and to bear two globular swellings that there is reason to regard as apogamous buds in an early stage. Each of these has characteristically altered tissue, with tracheides, and the upper bud had ramenta and a young leaf. It was found on studying the series that archegonia and antheridia were present in the immediate neighbourhood of the buds. These are not shown in the section figured except for one rather large and peculiar antheridium situated on the summit of the lower bud in close relation to its altered and more sporophytic tissue.

It remains to say something as to the sporangia themselves. Considering the position in which they are borne, these are remarkably normal in their development up to a fairly late stage. The main steps in segmentation have been seen; for example, stages with a three-sided pyramidal terminal cell; with a tetrahedral archesporial cell cutting off a tapetal layer that becomes two-layered; and with a group of sporogenous cells surrounded by the tapetum and differentiating capsule wall, were readily found. Very young sporangia are present on the prothalli represented in Pl. VIII, Figs. 7 and 8, and a more advanced example is given in Pl. VIII, Fig. 15. It is unnecessary, however, to illustrate the developmental stages in detail at present, although, if stages leading to mature sporangia with spores could be obtained in sufficient numbers, a complete critical comparison with the sporangia on the leaf of a sporophyte would become of interest. In the cases studied so far, the development follows a normal course up to a fairly late stage, stalk and capsule being differentiated, but the contents of the capsule disappear and it is usually flattened and empty or filled with air, no spores having been developed. This is the condition of the larger sporangia shown in Pl. VIII, Fig. 14, and explains the white appearance of the sporangial group represented in Pl. VIII, Fig. 2. An empty sporangium is represented more highly magnified in Pl. VIII, Fig. 16, to show the general differentiation of the annulus and lateral wall of the capsule.

Only a few examples of sporangia with the walls of the annulus brown and thickened and containing brown-walled spores have been met with.

Unfortunately they were all utilized for unsuccessful attempts to isolate and grow the spores, and no figure of this stage can be given. In one poorly stained example of such a sporangium met with in the study of sections, the spores appeared abnormal, with no contents and collapsed walls, but their arrangement suggested that they were grouped in tetrads. The material has, however, been insufficient for the study of this interesting question. The group of sporangia in Pl. VIII, Fig. 14, shows the typically mixed arrangement, young sporangia springing up between the stalks of the old ones and leading to the development of the crowded sporangial groups.

Discussion.

In the present state of our knowledge there is little use in attempting any full discussion of the phenomena presented by this Fern. It would have to be general, and include the consideration of other deviations from the normal course of the life-history of the Fern. There is no reason to think that a repetition of such general discussions would be fruitful until further facts are available.

Although the normal life-history has been known in all essentials since the middle of last century and has been investigated repeatedly in diverse examples of Ferns, a little reflection shows how unable we are to pass from a purely descriptive account to any comprehension of the causal factors involved in the developmental changes. These include the conditions determining the development of antheridia and archegonia and probably conditions in the prothallus that are requisite for the development of the embryo from the fertilized egg. It is of course true that this statement of the limitation of our knowledge to a description of successive stages reached in development applies to practically every developmental process and its results, with a few partial exceptions that only bring out more clearly the difficulty of advance to a causal understanding of morphological phenomena. With reference to the particular case of the life-history of the Fern I may refer to the treatment of the subject in works on Plant Physiology. It is clear from such accounts that all that can be done, even when special attention is being paid to this department of physiology (1), is to state the observed facts, mentioning such deviations from the normal as apogamy and apospory; and to express the hope that the conditions for the normal rhythm of alternation of generations may ultimately be discovered and the process altered at the will of the experimenter.

So far, however, though numerous cases of these deviations from normal development have been studied, they have only added to our knowledge of alternative processes of development that can be described but as little explained as the normal development. Without entering into details of the facts that have been repeatedly summarized, it may be recalled that many cases of apogamy and apospory are clearly dependent on the

inherited constitution of particular varieties or species of Ferns. These cases can be distinguished as direct apogamy or apospory. On the other hand, and of greater interest from the present point of view because more hopeful for analysis, are the instances that have been met with occasionally in a number of Ferns the inherited constitution and usual behaviour of which is apparently normal. We do not understand the causes leading to the occasional departures from normal development in such cases, but they can conveniently be distinguished as induced apospory and induced apogamy.

It is induced apogamy that specially concerns us here. A considerable number of cases of this, exhibiting diversity in the details of their departures from normal, are now known. When conditions allow of fertilization the prothalli produce young plants freely. It is prothalli that have passed the earlier stage at which fertilization most readily takes place and have usually attained considerable size that tend to exhibit departures from normal. In such unfertilized old prothalli continued growth, changes of form of the prothallus, or peculiar growths of the sexual organs are all that take place in many Ferns. In others this condition may pass on to some manifestation of apogamy. There may be the appearance of tissues or organs usually characteristic of the sporophyte or fully organized young plants may be apogamously developed. Another and peculiar manifestation of induced apogamy is the production of sporangia on the prothallus.

The three Ferns, two varieties of *Scolopendrium* and one of *Nephrodium dilatatum*, some of the prothalli of which have produced undoubted and well organized sporangia, all exhibited other manifestations of induced apogamy. In all three apparently normal embryos and young plants were produced when conditions facilitated fertilization. The further developments in old cultures of unfertilized prothalli exhibit considerable differences in detail. It is important to realize these differences, especially those that concern the way in which the sporangia are borne, since they increase the difficulty of making general inferences from the facts so far ascertained. The chief features of the three cases may therefore be summarized.

In prothalli reared from a plant of *Nephrodium dilatatum*, var. *cristatum-gracile* (4), which was in the collection of the late Mr. Druery, sporangia were often formed from the apical region; this then included tracheides and corresponded to the middle lobe of other apogamous prothalli. They also occurred in small groups on the cylindrical process, sometimes on the upper side and sometimes below. The cylindrical processes in this variety usually grew from below and just behind the anterior margin, but less commonly continued the growth of the prothallus. Such cylindrical processes bore sexual organs, usually normal-looking archegonia but sometimes groups of malformed sexual organs. Under certain conditions they could grow on into flattened prothalli. On the other hand they developed tracheides internally at the regions where the sporangia, sometimes asso-

ciated with ramenta, were borne. At the time the published description was written no apogamously produced plants had been observed, but one case of the production of such a sporophyte from the tip of a cylindrical process was met with subsequently. The sporangia on the prothalli of this Fern attained very complete mature structure, but no evidence to show that the spores were capable of germination was obtained.

The old prothalli reared from a plant labelled *Scolopendrium vulgare*, var. *ramulosissimum* (4), growing in the Royal Botanic Gardens, Kew, sometimes exhibited very irregular and thickened growths. Cylindrical processes, bearing numerous archegonia, were most commonly produced as a direct continuation of the apex, though they sometimes arose from the lower surface. The tip of such a cylindrical process sometimes became transformed into the shoot of an apogamous plant. Isolated ramenta and isolated roots were met with. The latter arose endogenously in relation to a vascular strand in the cylindrical process. Sporangia of typical structure, but usually more or less arrested in their development, occurred in groups upon areas of small-celled tissue containing tracheides. In a few cases their position seemed to be related to altered archegonia. The position of the sporangia was on the upper surface of the thickened continuation of the prothallus, and in one case just behind a bud which occupied the tip of a definite cylindrical process.

The old prothalli exhibiting further developments of the variety of *Scolopendrium vulgare* described in this paper have their own special features. As the fairly numerous examples that have been available for study show, there is considerable uniformity as to the position in which sporangia are developed and in the position of apogamous buds, when these are formed. The sporangia originate from the superficial layer of both the upper and lower surface in a position some distance behind the anterior margin. The continuation of the prothallus in front of the sporangium-bearing region had developed before the sporangia arose and is probably altered in nature, although it retains prothalloid characters and may bear sexual organs. One or more apogamous buds may arise from, or near, the anterior margin or from slender outgrowths of it.

This abnormal further development of some of the old prothalli, which may have remained relatively thin or become thick and massive, results in a growth with a fairly definite morphology of its own. Its production has evidently followed its own rule and, like a normal development, should be open to causal analysis. Such analysis would in some ways appear to be more hopeful in the case of the abnormal, though regular, development, but so far little or no progress has been made in the case under consideration. All that can be done is to comment on some features presented by it that are of special interest and may have significance for a future explanation. Such tentative considerations must be based on inferences from the form

and structure of the abnormally developed prothalli; for no insight into the external or internal causal factors determining such developments has so far been obtained.

In the first place there is no reason to doubt that normal fertilization and the development of healthy plants produced in this way takes place in this variety of *Scolopendrium*. While it is true that fertilization has not been followed, nor the cytological facts ascertained, the experimental differences between the watered and protected cultures are almost conclusive. The facts ascertained for this case greatly strengthen the assumptions made in other cases regarded as induced apogamy. This capacity for normal reproduction is the distinctive feature of induced apogamy as contrasted with obligatory direct apogamy in Ferns. In the light of the effect of watering in the cultures of the Fern under consideration the suggestion of von Goebel (2), that some similar cases might be really obligatory apogamy with the development of diploid egg-cells, does not seem to apply.

Probably the plants so readily raised from cultures have been normally produced. The apogamously produced plants are much more difficult to rear. The question as to differences between the two is still under investigation.

In the old prothalli of this Fern the cylindrical processes or archegonial projections, which were such a feature of cultures of the other forms in which sporangia were developed on the prothalli, have been practically wanting. No evidence has been forthcoming to connect the changed growths in these prothalli with archegonia. The change seems to take place in the direct forward growth of the prothallus, and is presumably in some way referable to an alteration in the apical meristem. It has been pointed out that the change is rather abrupt, partly because it is emphasized by the presence of cells with thickened brown walls at the transition region. These are a marked feature of this case and at first suggested the idea that they might mark the place of action of some external cause. No evidence for this has been obtained, and it seems more likely that these cells may indicate some difficulty of adjustment between the new type of growth and the prothallial tissue just behind. The appearance of the abnormal further developments behind both apices of a dichotomous prothallus (cf. Text-fig. 4) appears to point to internal rather than external causal factors.

The change in the meristem has led to the development of a growth of a sporophytic type. Apart from the later production of sporangia and buds this is manifested by the vascular tissue and sometimes by the appearance of intercellular spaces. On the other hand, it is clear that the change is not complete and discontinuous, as it is in the embryo or even in apogamous buds. This is shown by the prothalloid appearance of the tissue of the region between the vascular strand and the apical margin of the pro-

thallus and the frequency of the development of archegonia in this region. There is no reason to think that an irreversible change has taken place in such cases; although not actually observed in this plant it is probable that prothallial growth might be resumed. The repetition of the series of changes in the specimen represented in Pl. VIII, Figs. 12, 13, is indirect evidence of this.

The condition of the prothalli, on which the early appearance of young sporangia has been observed, shows that the new growth is complete from the transition region to the margin before sporangial development takes place. It is also clear that apogamously produced buds are usually not initiated until after the sporangial development is advanced, and that they form at or near the anterior margin of the growth. The relation of this anterior margin to the region where the sporangia originate appears to some extent comparable to that between the 'middle lobe' and the place of origin of a bud in some cases of direct apogamy. When no bud forms the sporophytic change is only indicated by the development of tracheides. In some cases after such changed growth has taken place the bud forms on the 'middle lobe' which Von Goebel prefers to term a 'verkümmertes Blatt'. In the prothalli under consideration here it does not seem to make matters clearer to think of the altered anterior margin in this way. It would rather seem as if the imperfect expressions of apogamous buds on the margin (cf. Text-fig. 6) would better be termed arrested leaves.

In the prothalli under consideration here, as in the two examples described in 1898, the extraordinary phenomenon is the development, and up to a point the fairly normal development, of sporangia in a position and under conditions unlike those in which they normally occur. As organs that normally only appear towards the end of development of leaves of an adult sporophyte they would in some ways appear to be the most unlikely organs to be thus prematurely developed. In this case there are no other isolated organs of the sporophyte (ramenta, roots) such as were found in the previously described cases. Nor is there any indication here of a relation, either of corresponding position or of transformation, between the sporangia and the reproductive organs of the prothallus. The developmental problem presented to us is the origin of fairly normally constructed sporangia on a definite region of the changed growth of these prothalli.

So far as I know, the only explanatory suggestions that have been made regarding the phenomenon as shown in the earlier examples have been either to regard the sporangial groups with their underlying altered tracheidal tissues as a greatly reduced and shortened manifestation of an apogamous bud (Sadebeck, 6), or to regard the production of sporangia as an extreme case of 'Frühreife' (von Goebel, 2). The first way of formulating the facts does not convey any suggestion of explanation of the origin of such organs as sporangia. Von Goebel's expression, on the other hand,

would bring the phenomenon into line with a number of others that are perhaps better, though very imperfectly, understood, but there seem to be objections to it. It does not fit with the striking fact common to all the cases that the sporangia are developed apart from and preceding the origin of apogamously produced buds, and that there is no evidence that the leaves of the latter have any tendency to an early production of reproductive organs. This is particularly clear in the present case.

When the first cases of production of sporangia, associated with induced apogamy, were described in 1898 the discussion took the line of estimating their possible bearing on the question of the nature and origin of alternation of generations in the Ferns. This question, then being actively discussed, appears to-day not less interesting but more hopeless of solution. Even its clear statement is difficult, and it is apparent that there was a degree of formality and crudity about the alternative explanations put forward. The facts regarding deviations from the normal life-history are less simple than they appeared to be, and the possibility of basing any historical explanation of the origin of alternation of generations on them seems very doubtful. While this affects destructively the validity or usefulness of much of the theoretical discussion in my earlier paper, the facts as regards the occurrence of induced apogamy in old cultures of unfertilized prothalli of various Ferns and the production of sporangia on the prothalli of two varieties of distinct species remain as described and illustrated. The case of another variety of *Scolopendrium vulgare* described in the present paper is in all main respects confirmatory of the earlier observations, while adding details of its own.

The more immediate interest of such phenomena is their bearing on normal development, into the causal factors of which we have as yet practically no insight. As will be evident from the remarks, above the deviations from normal development are, so far, equally difficult to understand. The indications that in such a case as the present the deviations from the normal are in some way induced, doubtless in a sufficiently labile or susceptible strain, appears to be strengthened. That organs of complicated structure such as sporangia should be developed with a considerable degree of normalness, in a position and under conditions so unlike those in which they appear as ultimate structures on the leaves of the adult sporophyte is a surprising and unexpected phenomenon. It affords a striking case of the development of an organ out of its own place in the developmental sequence, and raises the question of the factors determining the development in a special way.

The difficulty, apparent in the case of the Fern dealt with in this paper, of doing more than describing the main features is an illustration of how far we are from any causal understanding of either the normal development or the deviations from it. As regards the latter a knowledge of more

instances may assist by increasing our acquaintance with the variety of manifestations the abnormal changes may exhibit. The account of another variety of *Scolopendrium vulgare* given here is a contribution to this. What is even more desirable is the discovery of some varieties showing induced apogamy like that described here, but in which the exceptional developments, under the required conditions, affect all or most of the prothalli in a culture instead of appearing sporadically. Such a form would enable the inquiry into the internal and external conditions and the relations between them to be made with more hope of success than is afforded by the cases known so far.

I wish to express my indebtedness to my laboratory assistant, Mr. E. Ashby, for his help in this investigation in various ways, including his care of the cultures and the preparation of the serial sections.

SUMMARY.

1. A mutant of *Scolopendrium vulgare* with ramo-digitate, suprasoriferous leaves is recorded as affording another example of the production of sporangia on Fern prothalli.

2. This Fern breeds true, and evidence is brought forward to show that, when watered from above, the young plants result from normal fertilization.

3. Occasional old prothalli in cultures in which fertilization has been prevented develop groups of sporangia both above and below, some distance behind the anterior margin. A vascular strand is present in the sporangium-bearing region. The anterior prolongation may bear archegonia. At its anterior margin apogamously produced young plants arise.

4. Some special features of this case of induced apogamy and the production of sporangia on a prothallus are discussed.

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EXPLANATION OF PLATE VIII.

Illustrating Professor William H. Lang's paper on a variety of *Scolopendrium vulgare* that bears Sporangia on the Prothallus.

(*All the figures are from untouched photographs.*)

Fig. 1. Longitudinal section of small prothallus, showing a normal young plant attached by a foot; an archegonium is present on the dorsal surface. × 50.

Fig. 2. Anterior end of a prothallus protographed from above as it grew in a culture. It shows a group of sporangia some distance behind the anterior margin; the latter has grown out as a cylindrical structure, the swollen end of which later developed an apogamous bud. × 14.

Fig. 3. Longitudinal section, showing in connexion the prothallial tissue of the cushion, a sporangial group and buds developed apogamously from the margin of the anterior prolongation. × 50.

Fig. 4. Longitudinal section through a prothallus, showing the tissue of the cushion, the region with numerous sporangia above and below, and vascular strand within, and the anterior prolongation: there is a marked development of cells with thickened brown walls at the transition region. × 40.

Fi. 5. Similar section through the sporangium-bearing region in another prothallus: the vascular strand has a well-defined sheath and immediately outside this comes the layer bearing the sporangia. × 50.

Fig. 6. Similar section of another specimen: in this the tissue below the sporangia consists of several layers and has irregular outgrowths. × 50.

Fig. 7. Longitudinal section, showing the transition from the thick prothallial cushion to the anterior prolongation: a group of young sporangia is seen below, and other sections showed their presence above also. × 100.

Fig. 8. Section of the corresponding region of another prothallus with a well-developed vascular strand and young sporangia both above and below. × 108.

Fig. 9. Longitudinal section through the same region of another prothallus which bore no sporangia; the vascular strand dies out as usual in the anterior prolongation, which in this case has intercellular spaces. × 60.

Fig. 10. The corresponding region of another prothallus without sporangia: the bulge of tissue including irregular vascular strands is more marked towards the upper side: the anterior prolongation is prothalloid with archegonia. × 50.

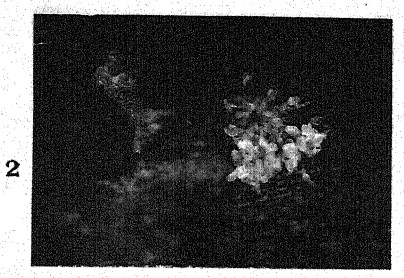
Fig. 11. Longitudinal section of the prothallus represented in Text-fig. 3, passing through the small group of sporangia borne on a projection of the upper surface. × 50.

Figs. 12, 13. Two longitudinal sections of a prothallus with two sporangial groups developed in succession, and apogamous buds on the anterior margin. Description in text. × 60.

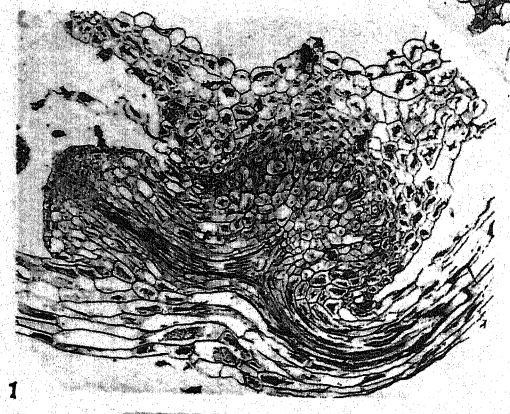
Fig. 14. Group of sporangia of mixed ages from a prothallus. × 150.

Fig. 15. Young sporangium with sporogenous cells and tapetum. × 300.

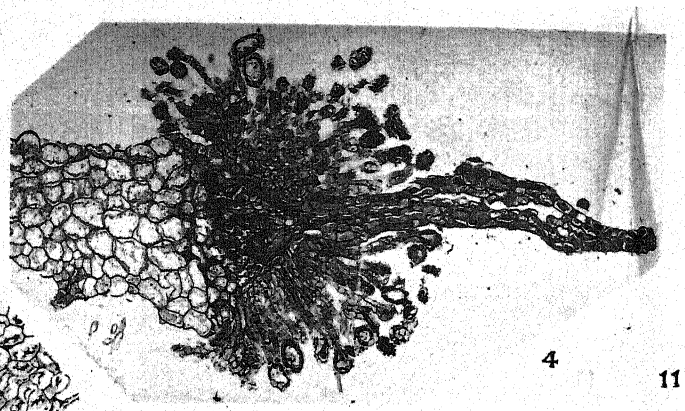
Fig. 16. Sporangium with well-developed wall and annulus, but arrested contents. × 300.



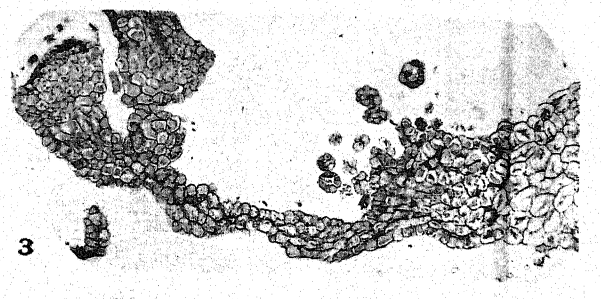
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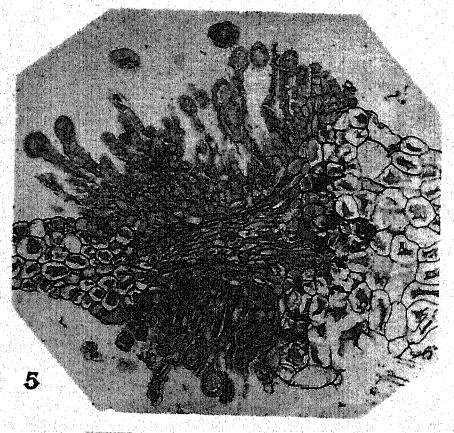
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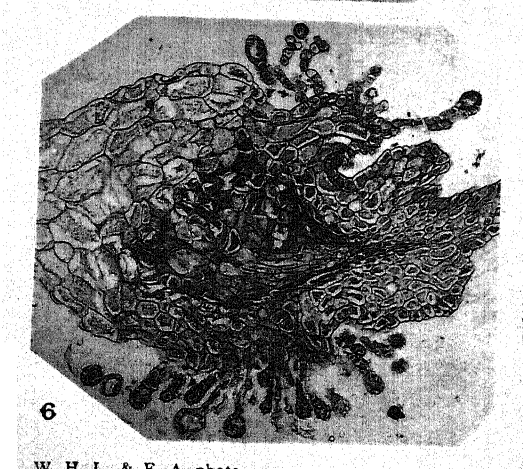
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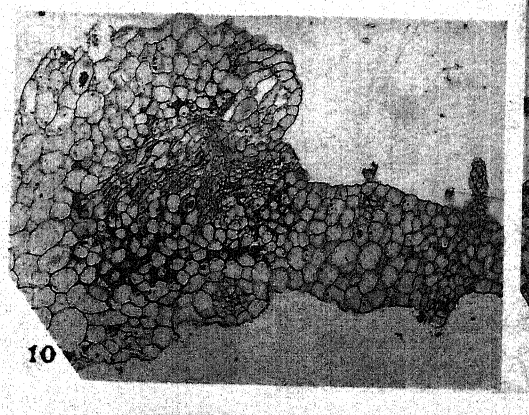
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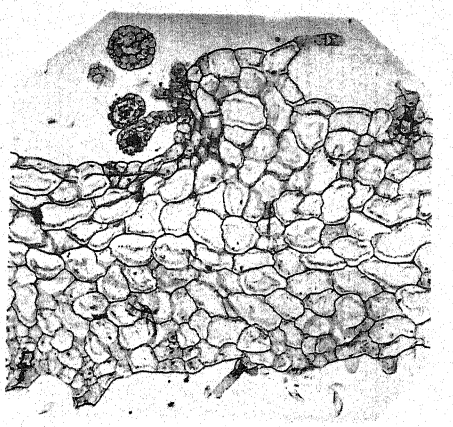
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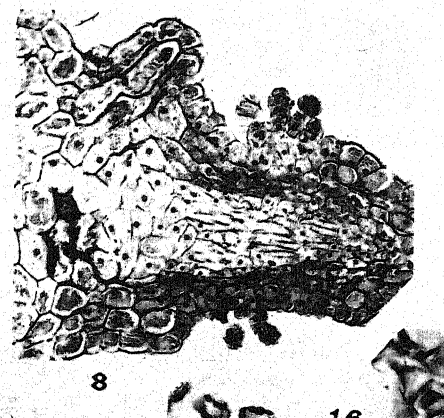
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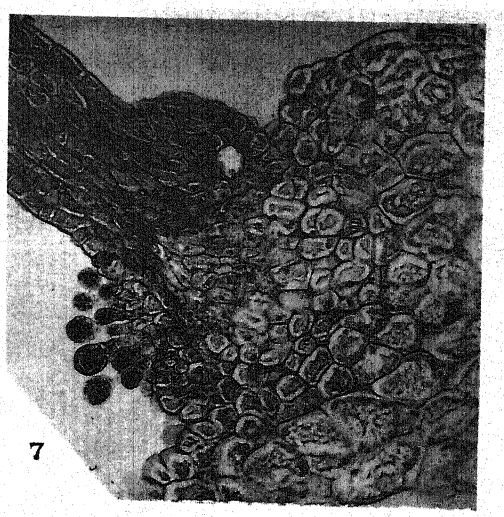
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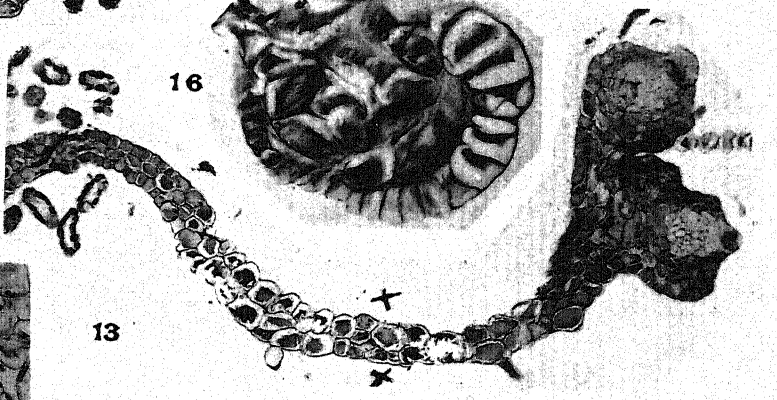


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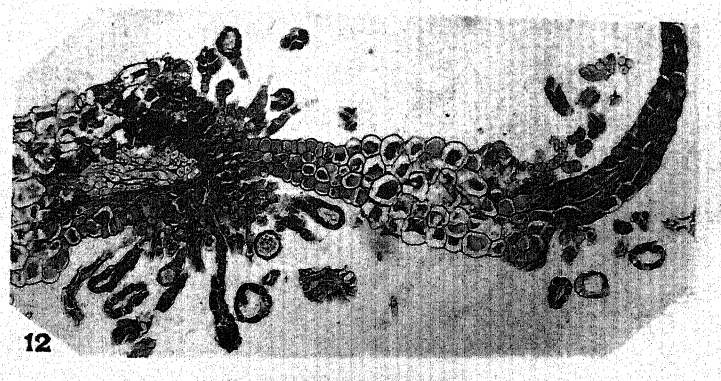


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W. H. L. & E. A. photo.

LANG — SPORANGIA ON PROTHALLUS.

Huth, London

Notes on Conifers.

I. The Older Fertile Ovule of Saxegothaea.

BY

W. T. SAXTON.

With three Figures in the Text.

THE structure of the young ovule of *Saxegothaea* became known in 1908–9 from the work of Norén (1908) and Tison (1908, 1909), and has also been illustrated by Stiles (1908, 1912). Norén figures an older stage of the ovule, but the prothallus there shown is completely abortive. Older stages in the development of the fertile seed appear to be quite unknown, and the present note may therefore be of interest.

Through the kind assistance of Professor J. Doyle the writer was able to collect material of this interesting Conifer from the garden of Kilmacurragh, Co. Wicklow, on July 18, 1928. Two cones were observed, in each of which one fertile scale was much swollen and bore a considerably enlarged ovule with a woody integument. The epimatium was quite short, reaching only about one-third the length of the ovule. On breaking open the integument it was found that the flaring apex of the nucellus had hardened and broken away from the nucellar cap, thus remaining like a stopper in the micropyle. The semi-diagrammatic drawing in Fig. 1 is from a sketch made at the time. An apparently healthy prothallus was found in each ovule, but sections prepared subsequently show that only one of them contained an embryo (Figs. 2 and 3). There are three features of interest noticeable in these figures:

(1) The narrow tube leading down to the top of the suspensor is much longer than in any other Conifer known to the writer. The appearance of very regular rows of cells radiating in a somewhat fan-like manner from a point at the base of the tube suggests that the latter may have been formed chiefly after fertilization.

(2) The suspensor is relatively short and massive, and bears a single embryo. There is no trace of cleavage polyembryony. Examination of the other sections of the series shows that no other embryo is present in the prothallus.

[Annals of Botany, Vol. XLIII. No. CLXX. April, 1929.]

(3) No trace of disorganized archegonia, such as might be expected to remain at this stage, can be seen. This fact rather suggests either a single

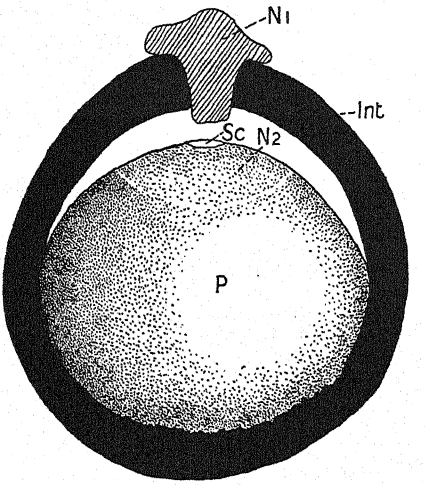


FIG. 1. Diagrammatic sketch of a radial view of the ovule, with the epimatium and half the integument removed. × 15. *N*1 = Nucellar apex; *N*2 = Nucellar cap; *Sc.* = Scar where *N*1 and *N*2 have separated; *P.* = Prothallus; *Int.* = Integument.

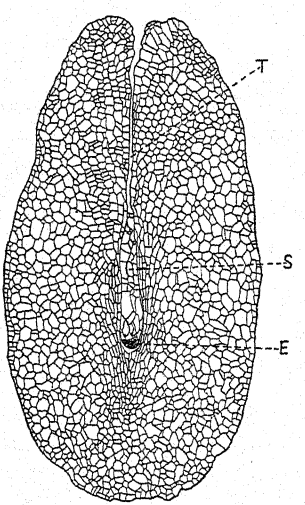


FIG. 2. Radial longitudinal section through the narrower diameter of the prothallus. × 27. *T.* = Apical tube leading to top of suspensor; *E.* = Embryo; *S.* = Suspensor.

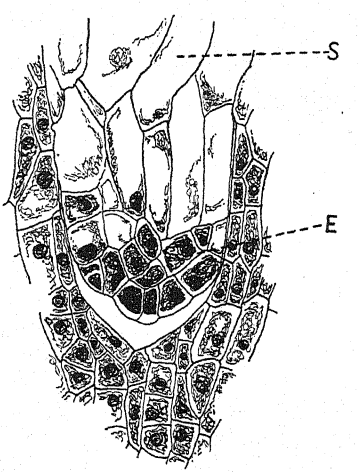


FIG. 3. Part of Fig. 2. × 220.

archegonium or a small apical complex such as occurs in *Microcachrys* (Lawson 1923). Further observations are needed to clear up this point, however.

It is worth noting that both the integument and prothallus are considerably flattened radially, so that the radial thickness is hardly more than one-third of the tangential diameter. Fig. 1 shows the latter, Fig. 2 the former.

The three figures were drawn by Miss L. E. Hawker, to whom my thanks are due.

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Studies in the Genus Fusarium.[1]

VII. Saltation in the Section Discolor.

BY

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With Plates IX and X and twelve Figures in the Text.

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I. INTRODUCTION.

IN an earlier paper (5) an account was given of the variation shown by certain species of *Fusarium* (section Discolor) when grown in nutrient media of varied composition. During the course of this work on variation, several saltants originating from *F. culmorum*, *F. polymorphum*, and *F. sulphureum* were obtained. These saltants showed marked divergence from the parent strains in certain respects, and particularly in spore shape, a character which has been regarded hitherto by systematists as relatively

[1] Thesis approved for the Degree of Doctor of Philosophy in the University of London.

stable. The object of the present paper is to give a detailed description of the saltants and their mode of origin, to compare them with the available Discolor species, and to indicate the bearing of this and the previous investigation (5) on the validity of the criteria used in classifying the species of *Fusarium*.

The various strains of *Fusarium* employed with the symbols used to denote them are given below:

Fusarium discolor App. et Wr. (Baarn) D
= *F. sambucinum* Fuckel
„ *sambucinum* Fuckel (Germany) Sa
„ *sulphureum* Schlechtendahl (Germany) SG
„ „ „ (Baarn) SB
„ *polymorphum* Matruchot (Germany) P
„ „ „ (Germany) P′
„ „ var. *pallens* Wr. (Germany) Pp
„ *culmorum* (W. G. Smith) Sacc. (Baarn) CB
„ „ (W. G. Smith) Sacc. (Imperial College) CJ
„ „ (W. G. Smith) Sacc. (Imperial College) CC
„ *lolii* (W. G. Smith) Sacc. (Baarn) L
„ *trichothecioides* (Amsterdam) T
„ *discolor* var. *triseptatum* (Baarn) Dtri.

The author is indebted to Dr. Wollenweber for the strains P, Pp, and SG, and also for identifying the strains CC and CJ.

For descriptive purposes the strains are grouped as follows:

discolor group	D, Sa, SG, SB.
polymorphum group	P, P′, Pp.
culmorum group	CB, CJ, CC.

As a rule the strains L, T, and Dtri will be referred to individually. Monosporous cultures were obtained before using any of the strains mentioned above for experimental purposes.

The media used were as follows:

(1) The Standard Medium (N)

Glucose	2 grm.
Potato starch	10 „
Asparagin	2 „
K_3PO_4	1·25 „
$MgSO_4\ 7H_2O$	0·75 „
Agar	15 „
Water	1 litre.

(2) Dilution Series. Altered concentration of the standard medium (2 N, N/2, N/4, N/10).

(3) Glucose Series. The standard medium with starch replaced by various concentrations of glucose (2G–20G viz. 2–20 per cent.).

(4) Cane-sugar Series as in (3) using cane-sugar instead of glucose (2G–20G).

(5) Asparagin Series. The standard medium with altered concentration of asparagin (0·1A–16A viz. 0·1–16 grm. per litre).

(6) Ammonium Tartrate Medium. The standard medium with asparagin replaced by ammonium tartrate.

(7) Acid-alkali Series. The standard medium with the addition of various percentage concentrations of acid (malic) and alkali (Na_2CO_3) — 1·5 Ac, 1·0 Ac, 0·5 Ac, 0·1 Ac, neutral, 0·1 Al, 0·5 Al, 1·0 Al.

II. A Comparative Account of the Available Species.

(a) *Rate of Radial Advance.*

The rates of radial advance in cm. per day in certain media for all the strains studied are given in Table I.

TABLE I.

Group.	Strain.	8G.	8C.	N.
Discolor	SG	0·85	0·77	0·53
	SB	0·75	0·97	0·66
	Sa	0·70	0·95	0·64
	D	0·80	0·92	0·62
Culmorum	CB	0·85	1·30	0·90
	CC	1·00	1·40	1·00
	CJ	0·90	0·95	0·70
Polymorphum	P	0·60	0·50	0·44
	P′	0·55	0·50	0·46
	Pp	0·65	0·62	0·51
	L	0·47	0·49	0·45
	T	0·60	0·75	0·52
	Dtri	0·70	0·97	0·60

It will be seen from the table that small differences in the rate of radial advance are shown by the strains included in each group. The three groups differ from one another mainly in relative vigour; the *culmorum* strains being the most vigorous, the *discolor* strains less vigorous, and those of the *polymorphum* group least vigorous. As pointed out in the previous paper (5) P and P′ alone show the staling character, but this character is not strongly developed. It will be shown in the section of this paper dealing with saltation that saltants derived from a given parent often show greater differences with respect to both the rate of radial advance and staling capacity than those which exist between any two strains given in Table I.

(b) *Mycelial Characters and Nature of the Sporing.*

The Discolor strains presented distinctive mycelial and sporing characters in all the media used. The chief features shown in standard medium plate-cultures eighteen days old are as follows:

Discolor group.

SG. Thin, uneven felt of moderately dense, dull-white mycelium with scattered, light cinnamon sporodochia. Dark brown sclerotia were present in cultures four weeks old.

SB. Thin, even, mycelial felt, pinkish buff owing to the presence of closely aggregated, minute, pink spore masses.

Sa. Clear centre surrounded by a thin mycelial felt. Large cream buff sporodochia are present; these are larger and more numerous as the margin of the culture is approached.

D. A thin, smooth felt of dense, white mycelium, often in two layers, the upper layer interrupted and only partially covering the lower. No sporing masses observed.

Polymorphum group.

P. Greyish white, loose mycelium. Large cinnamon sporodochia are present in the central region of the culture. Blue marginal sporodochia are formed later. Pl. IX, Fig. 5 *b*).

P′. Thin felt of moderately dense, white mycelium, sporodochia pale buff, minute, evenly distributed.

Pp. Copious development of dull white mycelium. Sporodochia not observed.

Culmorum group.

CB. Thin, white, mycelial felt bearing partially exposed, small, light salmon sporodochia over the whole surface.

CJ. Copious, woolly, vinaceous mycelium, bearing numerous, small, tawny olive, scattered sporodochia.

CC. Thin, pale pink mycelium with irregularly scattered, cinnamon sporodochia, these are smaller and less numerous in the direction of the margin of the culture.

Fusarium lolii.

L. An irregular, thin, white, mycelial felt with no obvious spore masses.

Fusarium trichothecioides.

T. Moderately dense mycelial felt, pinkish buff owing to the presence of very minute, densely aggregated spore masses.

Fusarium discolor, var. *triseptatum* Dtri.

A thin, smooth, dense mycelial felt showing no definite sporodochia.

When grown in the series of media 2N–N/10 the strains in general showed reduced aerial mycelium and decreased sporing capacity with increased dilution, at N/10 aerial mycelium being practically absent. A similar result was obtained with the asparagin series, in the direction of decreasing asparagin concentration.

The effect on the sporing is well shown in Pl. IX, Figs. 1–4 (Strain P). Minor differences between the strains as to the degree of mycelial development and sporing shown throughout any given cultural series were evident.

The colour of the spore masses in the various strains as observed after three months (asparagin series) is given in Table II.

TABLE II.

Strain.	Medium.				
		0·2A.	0·5A.	1A.	2A.
CC	Diamine brown.	Madder brown.	Cinnamon buff to madder brown.	Cinnamon buff.	
CJ	Dark brown.			Mummy brown.	
CB	Hyaline.		Pale ochraceous salmon.		
P	Dark purple.	Deep vinaceous purple.	Pale pinkish buff.	Pinkish buff.	Light cinnamon pink.
P′	Olivaceous to olivaceous black.				
Pp	Hyaline.				
D	Hyaline.			Cream.	Naples yellow.
Sa	Hyaline.	Pinkish buff.			
SB	Almost hyaline.				
SG	Hyaline.	Pale buff.			
L	Hyaline.				
T	Almost hyaline.			Cream buff.	

It will be seen from the table that the various strains in cultures of equal age differ in the colour of the spore masses. With some strains the sporodochia change in colour with age or the later formed sporodochia differ in colour from those formed earlier. Thus with the strain P, in cultures about a month old (0·5 A and 2 A) the central region is occupied by buff or cinnamon sporodochia. As growth proceeds these are surrounded by a narrow zone of bright blue sporodochia which darken with age, and

after three months are almost black. With P′, sporodochial development and the greenish (olivaceous) colour recorded in Table II are late developments. These sporodochia were at first yellow. The presence of the green coloration suggests the combination of the yellow with a blue pigment presumably developed later. Green sporodochia are not of uncommon occurrence in the genus *Fusarium* (e. g. *F. javanicum*). The occurrence of blue sporodochia has long been known for *F. coeruleum*. This blue colour is recorded by Sherbakoff (6, p. 245) for *F. martii* and *F. solani* var. *cyanum* (neutral media). It was also noted for *F. subpallidum* by Sherbakoff (6, p. 231) who remarks that it is an exceptional colour for an organism of the section Discolor and observed only in this species.

(c) *Substratal Colouring.*

The substratal colouring shown by the different strains when grown in the dilution, asparagin, and glucose series of media is recorded in Tables III, IV, and V. Except where a certain colour is said to be restricted to a particular region of the plate, it should be regarded as being uniformly distributed in the substratum.

TABLE III.

Colour Development in the Substratum—discolor Group.

Asparagin Series.

Strain.	2A.	A.	0·5A.	0·2A.	0·1A.
D 3 months	Absent	Pale vinaceous margin.	Pale vinaceous.	Absent.	Absent.
Sa 3 months	Bordeaux marginal zone.	Bordeaux	Pomegranate purple.	Hellebore red margin.	Hellebore red.
SB 3 months	Absent.				
SG 3 months	Absent.				

Dilution Series.

Strain.	2N.	N.	N/2.	N/4.	N/10.
D	As with asparagin series.				
Sa 5 days	Absent.	Vinaceous.			
„ 35 days	Absent.	Warm buff centre and dull Indian purple margin, decreasing in intensity from 2N to N/10.			
SB 35 days	—	Buff pink, decreasing in intensity from 2N to N/10.			
SG 15 days	Absent.				

Glucose 8 %.

Strain.	
D 15 days	Naples yellow to buff yellow.
S 15 „	Yellowish with brick-red marginal zone.
SB 15 „	Cream.
SG 15 „	Straw yellow to Naples yellow.

TABLE IV.

Colour Development in the Substratum—polymorphum Group.

Asparagin Series.

Strain.	2A.	1A.	0·5A.	0·2A.	0·1A.
P 12 days	Dahlia carmine.				
3 months	Very little change.				
P′ 12 days	Vinaceous marginal zone.	Hellebore red centre and margin honey yellow.	Hellebore red.		
3 months	Very little change.				
Pp 3 months	Buff.				

Glucose 8 %.

P 15 days	Burnt lake with pomegranate purple margin.
P′ 15 days	Mars yellow.

TABLE V.

Colour Development in the Substratum—culmorum Group.

Asparagin Series.

Strain.	2A.	1A.	0·5A.	0·2A.	0·1A.
CB 5 days	Absent.	Vinaceous increasing in intensity from 1A to 0·2A.			Similar to 0·2A.
3 months	Rhodonite pink marginal zone.	Hellebore red increasing in intensity from 1A to 0·2A.			Indian red.
CJ 5 days	Yellow with pink or red centre decreasing in intensity from 2A to 0·2A.				
3 months	Resembles CC but more intense at 2A.				
CC 5 days	Pale pink with maximum colour at 0·5A.				
3 months	Hellebore red marginal zone.	Neutral red (1A) to Indian purple (0·1A).			

Dilution Series.

Strain.	2N.	N.	N/2.	N/4.	N/10.
CB 6 days	Very slight.				
3 months					
CJ 26 days	Hessian brown.	Principally brick red.		Light buff; margin Pompeian red.	Light buff and eugenia red.
CC 6 days	Pale orange near centre.	Pink near centre.	Pale pink.		

Glucose 8 %.

Strain.	
CB 15 days	Coral red and pomegranate purple.
CJ 15 days	Burnt lake.
CC 15 days	Coral red, Bordeaux centre.

Reference to the Tables III, IV, and V shows that closely related strains may vary considerably with regard to colour development. Thus Sa (*F. discolor*) shows strong colouring (asparagin and dilution series), D (*F. discolor*, Holland) a marginal light vinaceous region and SG no colour at all. Again P and Pp, strains of *F. polymorphum* show marked colour differences also CB (*F. culmorum*, Holland) and CC (identified by Wollenweber as *F. culmorum*). It should be added that all the colour producing strains exhibit individual differences with regard to the nature and intensity of the colouring, the time at which it appears and its distribution in the medium. With regard to the strains L, T, and Dtri excluded from the tables very little colour was produced.

(d) *Sporal Characters.*

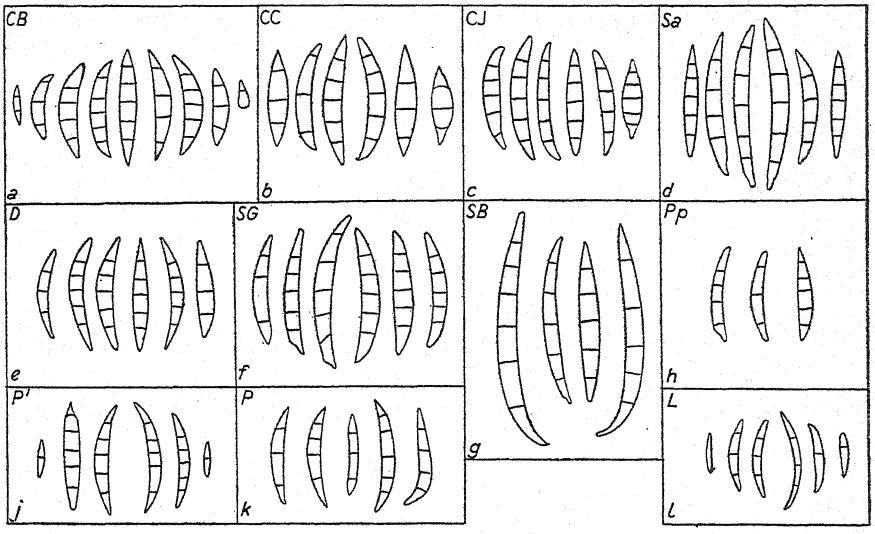
The spores of the three strains of *F. culmorum* (CB, CC, and CJ) resemble those described and figured by Sherbakoff for the species *F. culmorum*, *F. subpallidum*, and *F. clavatum*. With these spores the end segments are short and the septa somewhat closely disposed. The actual shape of the spore was not materially modified by varying the cultural treatment. Representative spores taken from cultures of CB, CC, and CJ on the standard medium are illustrated in Text-fig. 1 *a*, *b*, and *c* respectively.

The spores of the *discolor* group do not differ greatly from those of the *culmorum* group except that they are somewhat less abruptly attenuated at the ends as shown in Text-fig. 1 *d*. On certain media, however, spores are formed which are practically indistinguishable from those which characterize the *culmorum* group (Strain SG, Text-fig. 1 *f*). A variant of the type is found in SG and SB, where in certain media a certain proportion of the spores are more or less bent at one end.

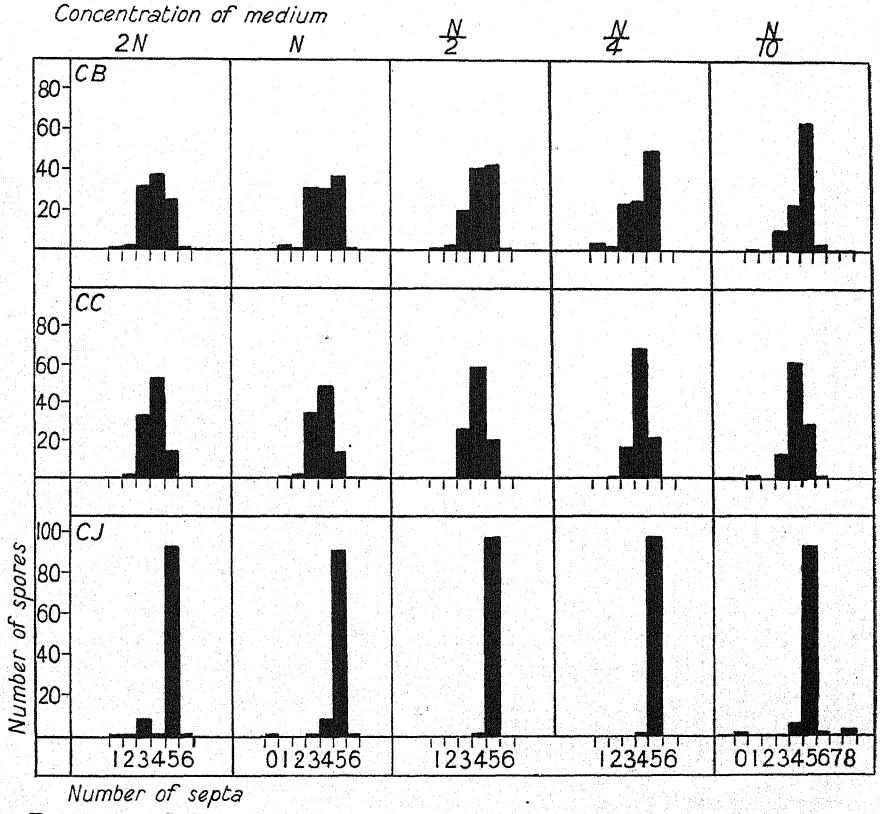
The spores of *F. polymorphum* Text-fig. 1 *h–k*, do not differ in any marked degree from those of the *discolor* group.

With regard to the remaining strains studied, in L (*F. lolii*) the spores are short and differ from those formed by the strains considered above in rarely showing more than three septa, Text-fig. 1 *l*. In T (*F. trichothecioides*) long, sickle-shaped spores were present in certain media; mixtures of long and short spores in other media, while in some cases, for example, standard medium cultures, only short spores were present. With Dtri typical sickle-shaped spores were of rare occurrence.

The septation of the spores and the effect produced on the septation and length of the spores by altering the composition of the medium has been dealt with in some detail in the previous paper (5). Further comments are necessary only from the point of view of making the comparative work more complete.

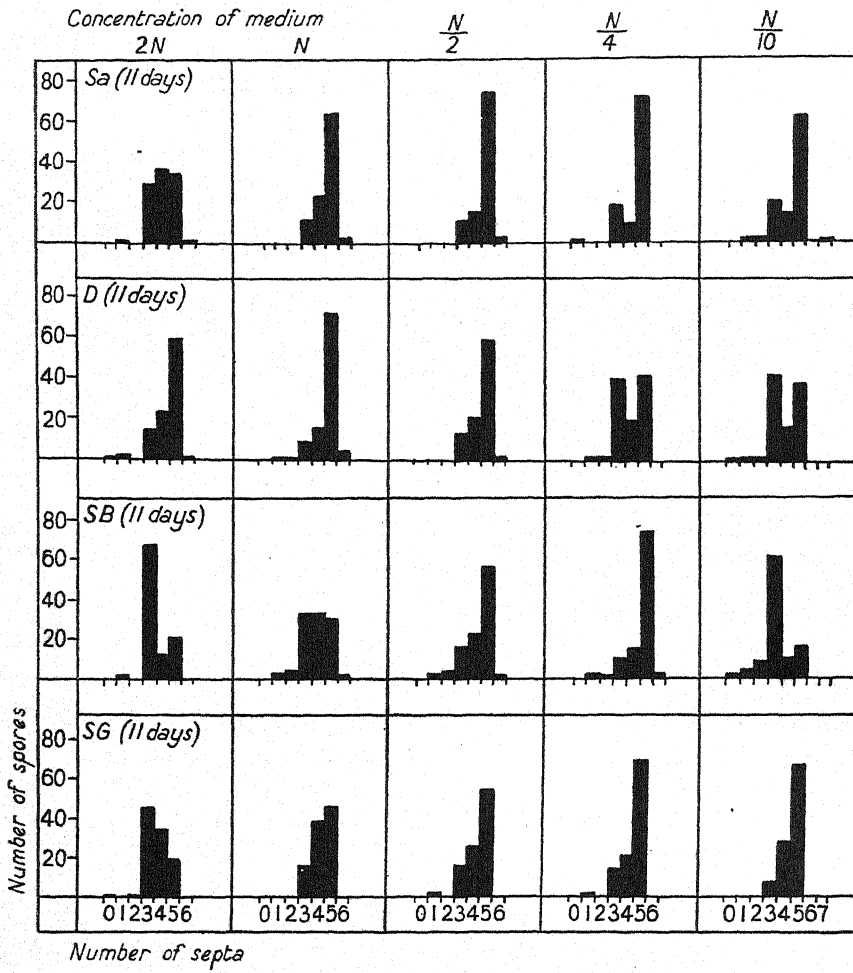


TEXT-FIG. 1. Spores of various Discolor strains.



TEXT-FIG. 2. Series 2N–N/10. Graphical representation of the distribution of septation as shown by the strains CB, CC, and CJ.

Culmorum group. The septation changes throughout the dilution series are shown in Text-fig. 2; with CB a low 4-mode is present at 2 N, whence the septation rises, culminating in a high 5-mode at N/10; with CC

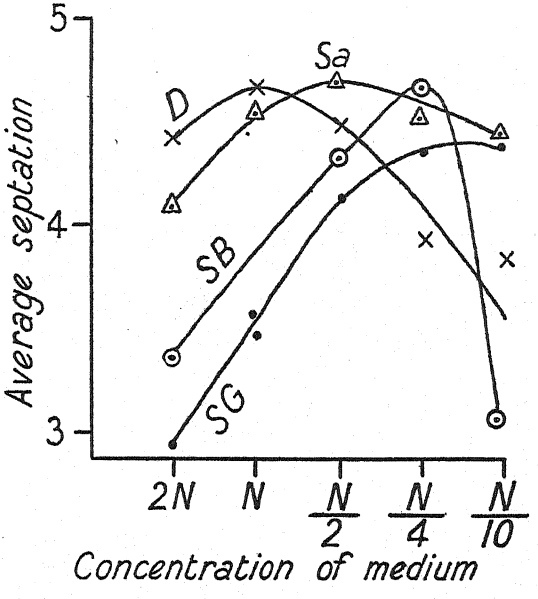


TEXT-FIG. 3. Series 2N–N/10. Graphical representation of the distribution of septation as shown by the strains Sa, D, SB, and SG.

a 4-mode, and with CJ an exceptionally high 5-mode, is maintained throughout the series. The three strains show 5-modes throughout the asparagin series (see reference 5, Fig. 16) differing from one another in certain details. With the glucose series (see H. and M., Table IV) CB and CC show a 5-mode at 2 G, the septation declines with increasing glucose concentration culminating in a high 3-mode at 18 G. CJ, on the other hand, shows a high 5-mode from 2 G to 20 G, but spores are rare at the highest concentration

CB and CC also differ from CJ in producing a certain percentage of short spores of low septation.

Discolor group. The strains exhibit marked differences with respect to

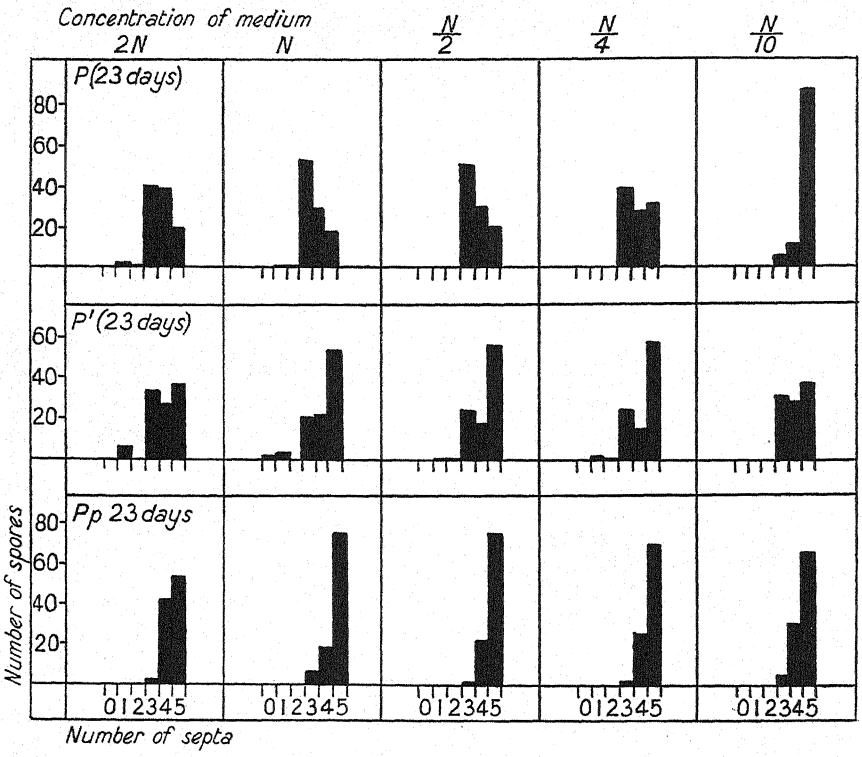


TEXT-FIG. 4. Series 2N–N/10. Graph showing average septation curves for the strains Sa, D, SB, and SG.

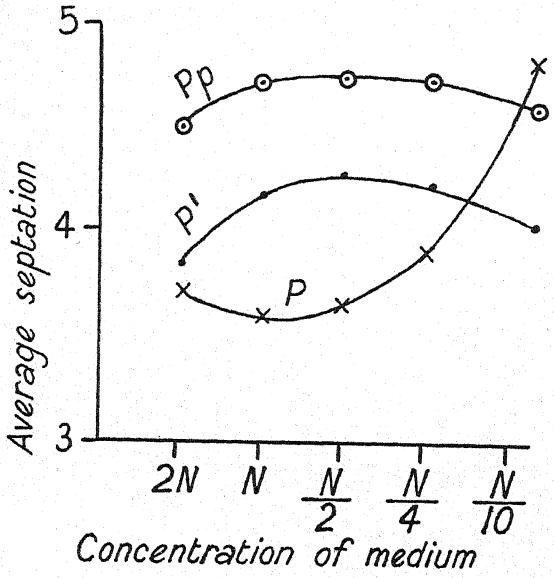
both the distribution of septation and the average septation when grown in the dilution or the asparagin series—see Text-figs. 3 and 4, also H. and M., Text-fig. 12.

With regard to glucose the four strains differ as to the glucose concentration limiting spore production: thus with SG and D practically no spores were formed at 8 G, with Sa a few spores (0–5 septate) were present, but with SB only short spores of low septation occurred at this concentration. These strains differ from those of the *culmorum* group in their inability to form spores at high glucose concentrations.

Polymorphum group. The graph for the distribution of septation in the dilution series given in Text-fig. 5 shows the difference between the three strains: Pp is markedly distinguished from P and P′ in showing a relatively high 5-mode throughout the series. The three strains show individual differences with regard to the average septation (Text-fig. 6). The type of curve shown for P, which is based on a septation count of 1600 spores, is exceptional and needs further investigation. A curve of a similar type is shown for *F. trichothecioides* in the previous paper (H. and M., Fig. 19). It will be seen on comparing Text-fig. 6 with Text-fig. 4 that no salient difference in septation separates *F. polymorphum* from the *discolor* group of strains.

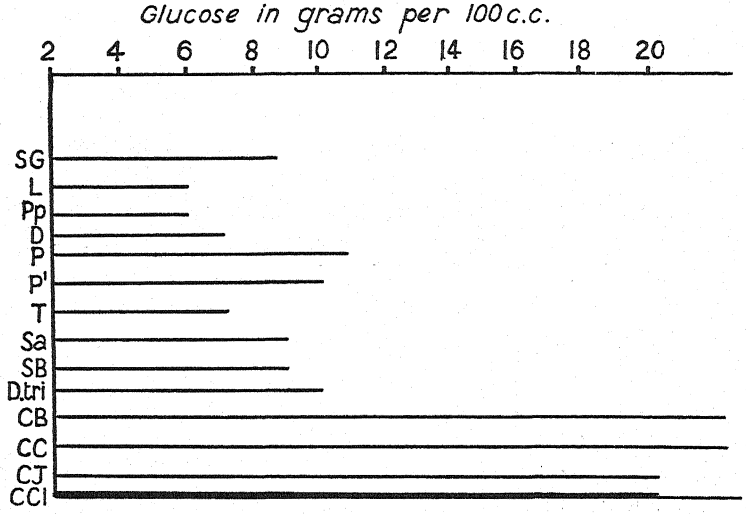


TEXT-FIG. 5. Series 2N–N/10. Graphical representation of the distribution of septation as shown by the strains P, P', and Pp.



TEXT-FIG. 6. Series 2N–N/10. Graph showing average septation curves for the strains P, P', and Pp.

The strains vary as to the glucose concentration limiting spore production (see H. and M., p. 530), and in common with the *discolor* group are unable to form spores at high concentrations. The limiting glucose concentration for spore formation for all the strains under investigation is shown graphically in Text-fig. 7.



TEXT-FIG. 7. Graph showing spore formation in relation to glucose concentration for certain strains.

The strains L, T, and Dtri have been studied in detail in the dilution series only. The spores of L are short and not more than 3-septate. There is a steady fall in the number of 3-septate spores and in the average septation from 2N to N/4 until at N/10 very few spores are formed and these are nearly all 0-septate (see H. and M., p. 525, Fig. 3). With T, at 2N and N a 1-mode is evident (short spores), which is replaced by a 3-mode at N/2 where a mixture of short and long (sickle-shaped spores) occurs, while at N/4 and N/10, where only spores of the second type are found, there is a 5-mode (see H. and M., p. 525, Fig. 3). The strain Dtri stands apart from all the others studied in exhibiting a high 0-mode throughout the dilution series. Numerous pear-shaped spores were also present (see *F. sporotrichioides*).

As a general conclusion it may be stated that the strains of *F. culmorum* exhibit more stable septation under cultural treatment than those of the remaining species dealt with in this paper.

Chlamydospores, in both terminal and intercalary chains and sometimes in groups, are of general occurrence in the section Discolor. The strain CJ is exceptionally prolific in this respect, the ferruginous appearance

shown by old cultures being almost entirely due to innumerable brown, warty chlamydospores as in the case of *F. ferruginosum* (6, p. 190).

III. A Comparative Account of the Saltants.

(*a*) *Fusarium sulphureum.* Schlechtendahl. SG, SB.

Saltants were obtained only from SG. This strain, which was received from Germany in June 1925, did not show any signs of sectoring on the standard medium (N). On December 21st accordingly, using Brown's method (1), a hyphal tip was transferred from a growth on a plain agar plate to a plate of Richard's solution agar, and this plate was kept inside a larger plate at room temperature to prevent contamination. On Feb. 1st, 1926, when the culture was about forty days old, inocula taken from different parts of the culture were transferred to standard medium plates. In one of these plate-cultures a strain appeared which differed from the parent, SG, in forming numerous, small, evenly distributed sporodochia and in the nature of its mycelial surface. This new strain was designated SG 1. Another of the plate-cultures derived from the Richard's culture showed distinct sectoring (Pl. X, Fig. 6). The larger sector, which occupied about two-thirds of the plate, showed a milk-white, dense mycelium, and the smaller sector a loose, greyish-white mycelium. Inocula transferred from the two sectors to standard medium plates gave rise to cultures resembling the parent sectors, and in this way two more new strains (with white mycelium, SG 2; with grey mycelium, SG 3) were obtained which were markedly unlike each other and differed considerably from the original parent (SG).

About the same time sectoring was observed in standard medium plate-cultures where ammonium chloride had been substituted for asparagin. The cultures (see Pl. X, Fig. 7) showed two dissimilar sectors—a clear non-mycelial sector occupying the greater portion of the plate and a small mycelial sector consisting of loose, white, erect mycelium. Subsequently cultures of two kinds were obtained, one kind showing a clear non-mycelial surface (SG 4), the other a mycelial surface (SG 5), both of which were unlike the parent.

Thus in all, six apparently different strains, including the parent, had been obtained. On growing these strains for comparative purposes in various media it was ultimately found that SG 5 consisted of a mixture of SG 1 and SG 3, either the one or the other showing itself as a sector in plate-cultures. This circumstance explained a fact that in making septation counts SG 5 gave, in some cases, a 5-mode and in others a 3-mode when grown in the same medium. The four remaining saltants have been tested several times in different media and have been found to preserve their individual characteristics, and they differ also from the parent strain.

The mycelial and sporal characters of the five SG strains (parent and saltants) as observed in plate cultures (N) seventeen days old), are compared in Table VI.

TABLE VI.

Characters shown by the Strain SG and its Saltants in the Standard Medium (Seventeen Days).

Strain.	Mycelium and Sporodochia.	Sclerotia.	Spores.	Average Septation.	Spore Mode.
SG	Thin, felt-like; scattered light cinnamon sporodochia.	Present	Frequently bent.	4·31	5-mode.
SG1	Thin, felt-like, with closely set, cinnamon-buff sporodochia.	Absent	Fairly uniform, 52 μ long.	4·46	5-mode.
SG2	Dense, white, slow-growing with minute spore masses.	Absent	Clear to subgranular not uniform.	3·30	3-mode.
SG3	Loose, greyish-white with marginal cinnamon sporodochia.	Present	Not uniform.	2·70	3-mode.
SG4	Marginal only: sporodochia few, pinkish buff.	Absent	Uniform, subgranular, 64 μ long.	4·88	5-mode.

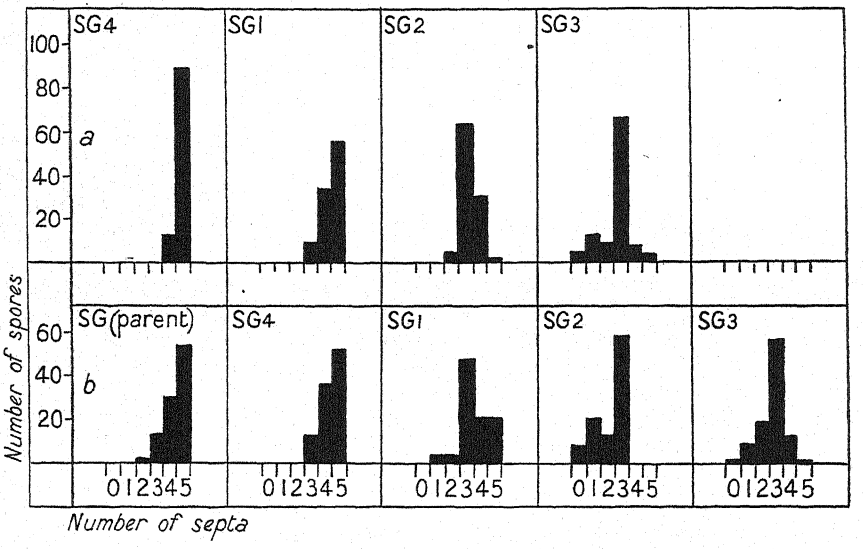
SG 2 is distinguished from all the other strains by its white, dense mycelium and the absence of definite sporodochia, and in showing the staling type of growth. The greyish-white, loose, mycelium in SG 3 and the smooth surface in SG 4 are also characteristic. With regard to SG and SG 1, the uneven mycelial surface and the large scattered sporodochia of the former serve to distinguish it from the latter, which has a very smooth, even mycelial surface and smaller closely set sporodochia.

Only two of the strains, SG (Pl. X, Fig. 8) and SG 3, formed sclerotia, but these do not usually make their appearance until the cultures are from four to five weeks old. The sclerotia are dark-brown and from 2 to 4 mm. in diameter.

With regard to length and shape, the spores of SG 1 and SG 4 are fairly uniform, those of SG 4 being longer and more uniform than the spores of the remaining strains. The spores of SG 2 and SG 3, on the other hand, are not uniform, varying both in length and shape. The strain SG 3 is noteworthy from the occurrence of conidia of the arthrosporal type.

The degree of uniformity in length and shape is reflected in the septa-

tion (Text-fig. 8). In standard medium cultures (Text-fig. 8 *a*) SG 4 shows a high 5-mode, and nearly all the spores are 5-septate. In SG 1 the average septation is lower, but there are no spores of low (0, 1, or 2) septation. With



TEXT-FIG. 8. Graphical representation of the septation as shown by the saltants of *F. sulphureum*.

SG 2 and SG 3, on the other hand, the lack of uniformity is associated with the presence of spores of low septation. The saltants show the same but somewhat lower modes in ammonium tartrate medium cultures (Text-fig. 8 *b*).

Taking into account the characters described above, the saltants may be classified as follows:

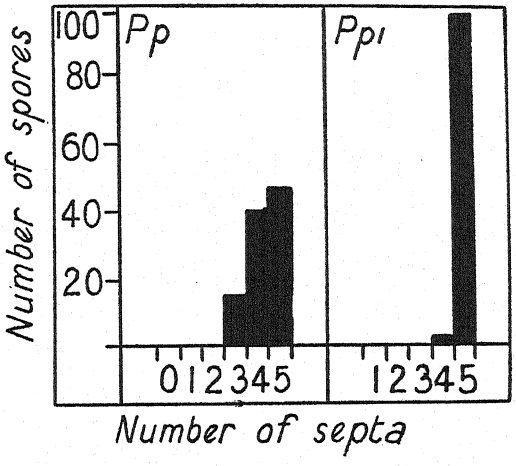
A. Spore masses confluent
Spores relatively long and of uniform shape
5-mode . . . SG 4.

B. Sporodochia prominent
Spores less uniform than A
5-mode . . . { SG sclerotia present.
SG 1 sclerotia absent.

C. Sporodochia not prominent
Spores not uniformly long
3-mode . . . { SG 2 sclerotia absent.
SG 3 ,, present.
Conidia of arthrosporal type present.

(*b*) *Fusarium polymorphum*, Matruchot.

Two strains, P and P′, were obtained on subculturing from the original culture obtained from Germany, but of these neither subsequently gave rise to saltants. Sectoring was observed, however, in the case of Pp (*F. poly-*



TEXT-FIG. 9. Graphical representation of the septation as shown by *F. polymorphum* var. *pallens* and the saltant Pp1.

morphum var. *pallens*) in the 2 A and 0·2 A media, the inocula used having been taken from a culture of monosporous origin. Using mycelial inocula, subcultures of the sectors bred true to the sectors, and in this way the saltant Pp1 was obtained. The saltant differed from its parent in mycelial characters and in its slower rate of growth.

On both the N and the ammonium tartrate media the mycelium of Pp was copious, spongy, and erect, whereas that of Pp1 was thin and felt-like. The colour of the mycelium was in both cases white, except that in the case of Pp on the ammonium tartrate medium it was a very pale yellowish white. On 18 G the differences in mycelial texture and colour were emphasized, Pp showing an erect, spongy mycelium with clay colour in the substratum, Pp1 developed a tough, leathery, vinaceous-cinnamon film, the substratum being cinnamon buff.

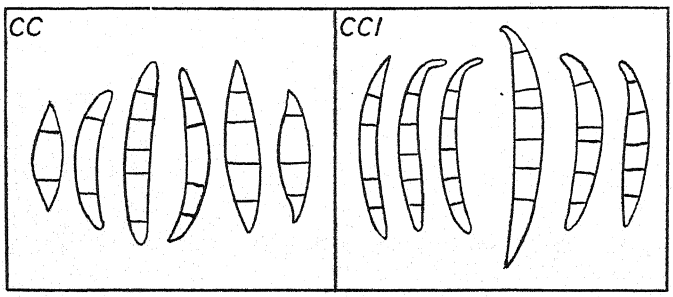
The spores of the saltant resembled those of the parent in shape. As to septation both strains showed a 5-mode, but the mode was much higher in the case of the saltant (Text-fig. 9). Chlamydospores were present in Pp, but were not observed in Pp1.

(*c*) *Fusarium culmorum* (W. G. Smith), Sacc. CB, CC, CJ.

Saltants were obtained only from CC and CJ.

Strain CC. This strain had been in culture at the Imperial College

since 1922 and did not show unusual features until June 1925 when several cultures were made, using single spores in each case. One of the monosporous cultures obtained in this way soon proved to be entirely unlike the parent, the new strain (CC 1) differing markedly from the parent in its pionnotal character.



TEXT-FIG. 10. Spores of parent and saltant strains of *F. culmorum*. (CC and CC 1).

The saltant proved to be a relatively slow-growing strain—0·43 as against 1·0 cm. per day in standard medium cultures.

With regard to substratal colouring, the parent strain showed vinaceous shades, hellebore red, Bordeaux red, &c., whereas colours were usually less intense or absent in the case of CC 1.

The spores of CC 1 are usually distinctly longer than those of CC and are frequently more or less strongly bent at one end (Text-fig. 10). Both CC and CC 1 are able to produce numerous spores at a glucose concentration of 20 per cent (20 G), and the distribution of the septation throughout the glucose series is practically the same for both strains. The curves for the average septation of CC and CC 1, in the asparagin series, show a distinct difference (see H. and M., Figs. 16 and 17).

Strain CJ. Although this fungus had been cultured in several different media since 1923, when it was isolated from a gooseberry fruit, no abnormal development of any kind had been observed until December 1925. On December 15th an experiment was made with certain strains, including CJ, using the glucose series of media. Duplicate series of plates were inoculated, using cultures of monosporous origin, and kept at 20° C. After a few days it was observed that the 16 G and 18 G plates presented unusual features.

With the two 16 G plates the culture in one of the pair was of the normal type, the other, however, showed marked differences in the rate of radial advance and in colour, the mycelium exhibiting a yellowish colour, and the substratum a difference in the time taken for the development of the colour characteristic of the original strain. It was at first thought that these differences might be due to variation in the composition of the medium, but on subculturing, repeatedly using the same medium, similarly prepared, the same differences were always present. Thus it was evident that a new

strain (CJ 1) had arisen. In standard medium cultures CJ 1 showed a thin white growth of aerial mycelium, and the substratal colouring was yellowish-buff fringed with dahlia carmine. On March 23rd, 1926, three N plates were inoculated with CJ 1. One of these plates soon showed a new development, the central portion of the plate exhibited an irregular growth of white mycelium (dense to almost absent), and the substratum underlying this region was almost colourless. The peripheral region, on the other hand, was occupied by pink mycelium, and the substratal colour was pink. Subcultures using mycelium from the central (white) and peripheral (pink) regions respectively were made in the ammonium tartrate medium. As a result strains of two distinct types were obtained. These strains, together with CJ and CJ 1 were then transferred to plates of 18 G for comparative purposes, and after a time it was found that the strain which had originated from the central white portion of the sectoring plate differed very considerably from both CJ and CJ 1. Plate-cultures of this new saltant (CJ 1, 1), using the ammonium tartrate medium, showed further sectoring, the sector taking the form of a raised circular ridge about 1 cm. wide and situated about 1 cm. from the centre of the plate. Subcultures, using mycelium from the ridge, gave rise to a new form as determined by the growth in 18 G (CJ 1, 1, 1), whereas others using mycelium from the margin, were identical with the parent (CJ 1, 1). Subsequently, on further subculturing (May 8th) CJ 1, 1 again sectored, giving rise to another new strain (CJ 1, 1, 2).

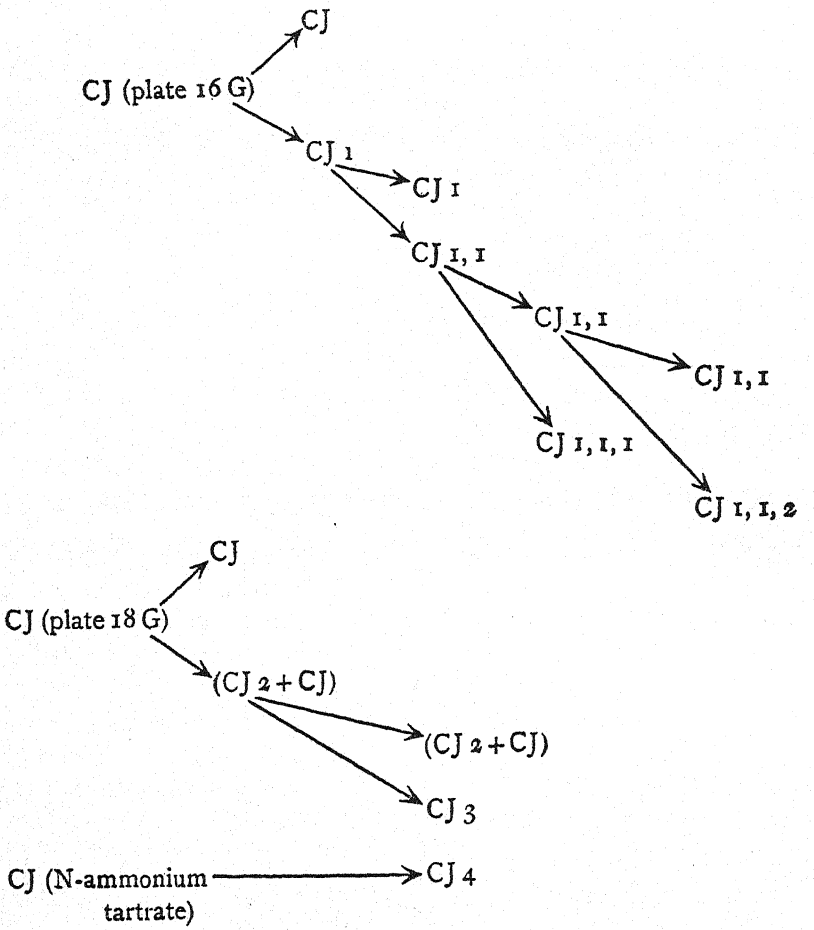
With regard to the two 18 G cultures, one was of the normal type, the other (CJ 2) was of a remarkable character. It differed from all the Discolor strains under investigation by the author in its strong capacity for staling and extremely wrinkled substratal growth, moreover the pink colour characteristic of the parent strain was absent. Subcultures were then made, using the surface mycelium taken at different distances from the centre, the substratal mycelium, &c., and as a result plate-cultures of different types were obtained.

Type 1. In general appearance the culture resembled the parent (CJ), growing rapidly and producing copious mycelium, but a well-defined patch of denser mycelium (CJ 2) was present in the centre, and on turning the plate upside down a corresponding patch was observed in the substratum (Pl. X, Fig. 9). Repeated attempts to obtain the slow-growing strain (CJ 2) in pure culture were unsuccessful.

Type 2. The cultures resembled type 1, except that dense mycelium was absent from the central region and replaced by a dull yellow slime (Pl. X, Fig. 10). Subcultures were then made by transferring portions of the slime material to plates of 18 G, and as a result growths were obtained which showed a smooth pionnotal surface and spores of the long-spore type (as observed on medium N). This very characteristic strain was designated CJ 3.

Further sectoring occurred when the ammonium tartrate medium was used. The sector which occupied rather less than one-third of the plate (Pl. X, Fig. 11) differed from the parent (CJ) in appearance, the mycelium being less vigorous and of a different colour. Subcultures using mycelium from this sector reproduced the characters of the sector. This saltant was designated CJ 4.

The origin of the various saltants is represented schematically below:



In all seven saltants have been obtained, of these one has not been separated, so that six remain to be dealt with. These saltants, together with the parent (CJ), have been grown in the N and 18 G media for comparative purposes. The chief results obtained are given in Tables VII and VIII.

It is clear from the tables that the saltants differ from the parent and amongst themselves in several important respects, notably in

1. Shape of the spores.
2. Spore development.
3. Development of atrophy.
4. Septation in relation to glucose concentration.
5. Chlamydospore development.
6. Rate of radial advance.
7. Substratal colouring.

TABLE VII.

Characters shown by the Strain CJ and its Saltants on the Standard Medium (Eight Days).

Strain.	Staling.	Colour and Nature of the Mycelium.	Colour of the Substratum.	Spores.	Average Septation.	Chlamydospores.
CJ	Absent	Copious, loose, erect, pale brownish vinaceous.	Corinthian pink with buff margin.	*Culmorum* type.	4·88	Numerous, terminal and intercalary.
CJ 1	Absent	Copious, loose, white to pale vinaceous lilac.	Light buff to vinaceous lilac.	'Hooked' type.	4·96	Numerous, terminal.
CJ 1, 1	Present	Dense white.	Naphthaline yellow; vinaceous purple margin later.	Spores immature.	—	Relatively few (8 days): extremely numerous (15 days).
CJ 1, 1, 1	Present	White, less copious than in CJ 1, 1.	Palenaphthaline yellow.	Extremely scarce (15 days).	4·49	Extremely numerous, immature (15 days).
CJ 1, 1, 2	Present	Moderately dense with thin centre.	Straw yellow; margin pale vinaceous.	Fusoid type.	3·41	Terminal and intercalary.
CJ 3	Present	Scanty, greyish white.	Absent, vinaceous margin later.	Elegans type, much atrophy.	5·07	Terminal and intercalary.
CJ 4	Absent	Grey white.	Absent, deep vinaceous margin later.	*Culmorum* type, 0–9 septate, variable, no atrophy.	4·36	Absent.

TABLE VIII.

Characters shown by the Strain CJ and its Saltants on the 18 *G Medium (Nineteen Days).*

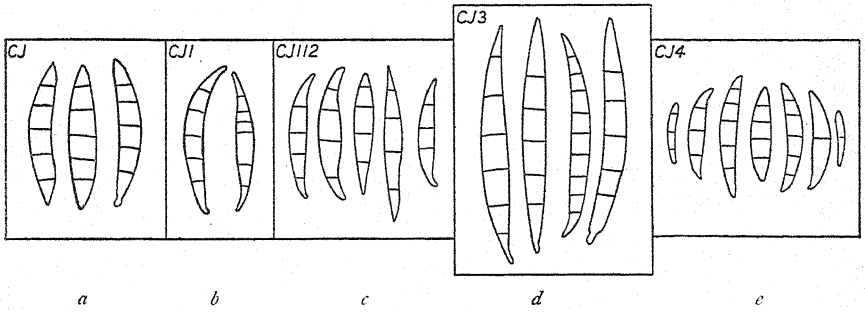
Strain	Staling.	Colour and Nature of the Mycelium.	Colour of Substratum.	Spores.	Average Septation.	Chlamydospores.
CJ	Absent	Copious, erect, greyish white tinged with pink and yellow.	Yellow	*Culmorum* type.	4·27	Very numerous.
CJ 1	Absent	Copious, erect, pale yellow with a broad white margin.	Aniline yellow.	Absent	—	Immature.
CJ 1, 1, 1	?	Dense white with yellow margin.	Chamois.	Absent	—	Few, immature.
CJ 1, 1	Present	Felt-like, pale yellow.	Chamois to honey yellow.	Absent	—	Absent.
CJ 1, 1, 2	Present	Loose, pale to naphthaline yellow.	Aniline yellow.	Very few, clear.	4·16	Very numerous.
CJ 3	Present	Drab grey: margin irregular.	Cream.	Irregular, highly vacuolate.	2·28 (8 days)	Very numerous.
CJ 4	Absent	Loose, fluffy, erect, pale vinaceous lilac.	Carrot red and honey yellow.	Numerous, granular.	3·34	Absent.

N.B. The data given in columns 3 and 4 were recorded after 7 days' growth.

(1) *Shape of the spores.*

As mentioned earlier in this paper, the spores of the parent strain (CJ) agree very closely with those described and illustrated for *F. culmorum* var. *leteius*, *F. subpallidum*, and *F. clavatum* by Sherbakoff (6, Figs. 39, 40 and 43). An example of a typical spore of the *culmorum* type is shown in Text-fig. 11 *a*. The spores of CJ 3 (Text-fig. 11 *d*), however, differ strikingly from CJ in that they are longer and narrower, and usually gradually pointed towards both the basal and apical ends, not abruptly attenuated as shown in Text-fig. 11 *a*, presenting a stronger likeness to those of *F. oxysporum* (section Elegans) than they do to any species belonging to the Discolor

section. The spores of CJ 1 are of the *culmorum* type, except that they are sometimes bent at the apical end (Text-fig. 11 *b*). This type of spore is also shown by CC 1, SG and SG 3. The spores of CJ 4 are also of the *culmorum* type, but differ from the parent in lack of uniformity. The spores of CJ 1, 1, 2 vary in shape and differ from those of CJ, in the presence



TEXT-FIG. 11. Diagram showing spore types in *F. culmorum*.

of more or less fusiform spores with tapering ends or spores with either the narrow apical end, or both the apical and basal ends somewhat bent (see Text-fig. 11 *c*).

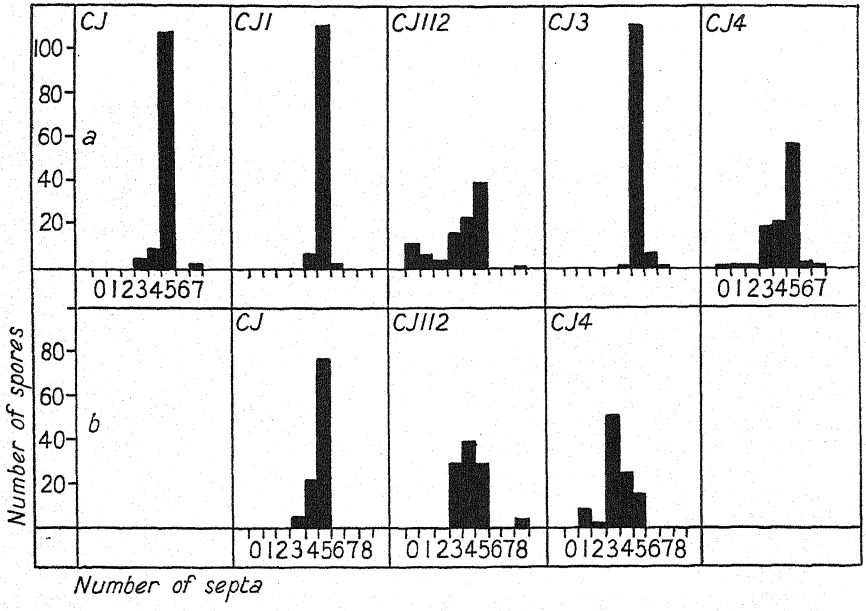
(2) *Spore development.*

It will be seen from Tables VII and VIII that the saltants vary in the degree of septation shown after a given interval of time (eight days). The septation shown by the strains which had produced spores in the standard medium is shown in Text-fig. 12 *a*. All the strains show a 5-mode, but vary in the strength of the mode. With CJ 3 when the spores have attained complete development—and development takes place earlier than with the remaining strains—the spores are nearly all 5-septate. With CJ 1, 1, 2 and CJ 4, on the other hand, the mode is low, and many spores show low septation. Such spores are of average size and are obviously immature. Hence it is probable that these strains, unless affected by atrophy, would ultimately show a high 5-mode. With CJ 1, 1 and CJ 1, 1, 1 very few spores were obtained in cultures.

(3) *Development of atrophy.*

With CJ 3, atrophy appears immediately the spores have become fully developed, thus in standard medium cultures ten days old, nearly all the spores are affected and sporal chlamydospores are formed. Hence in order to study the septation, the counts should be made on the eighth

day. With CJ 1, 1, 2 atrophy appears before the spores have attained their full development, and for this reason it is difficult to estimate the average septation accurately; if the record be made early (eight days) the average septation will be low owing to the prevalence of immature spores, if made



TEXT-FIG. 12. Graphical representation of the septation shown by parent and saltant strains of *F. culmorum*. *a*, standard medium; *b*, 18 G medium.

later it will be low owing to the development of atrophy. With the remaining strains atrophy was observed only in much older cultures.

(4) *Septation in relation to glucose concentration.*

A high concentration of glucose (18 G) is unsuitable for early spore formation. Observations made on the tenth day showed that only two (CJ, CJ 3) of the seven strains had produced spores. Later observations (nineteenth day) showed that three strains (CJ 1, CJ 1, 1, and CJ 1, 1, 1) had failed to produce spores. With CJ only a few were formed, these were of the clear type and showed a high 5-mode (Text-fig. 12 *b*). With CJ 4 and CJ 1, 1, 2, the high glucose effect was reflected in the septation CJ 4, showing a 3-mode and CJ 1, 1, 2 a 4-mode; the spores were shorter than those of CJ, and very short spores of low septation were present. The spores of CJ 1, 1, 2 were granular. With CJ 3, a strain showing early spore development and early sporal atrophy (standard medium), the spores are

relatively few, highly vacuolate and misshapen; they are small, 0–3 septate and difficult to distinguish from the segments of the fragmenting mycelium.

(5) *Chlamydospore development.*

With CJ, chlamydospores are present in great abundance after eight days. This character is retained by CJ 1, CJ 1, 1, 2, and CJ 3. With CJ 1, 1 and CJ 1, 1, 1, chlamydospore formation occurs later, and then nearly every portion of the mycelium becomes involved in producing them. In the case of CJ 4 very few chlamydospores have been observed in cultures.

(6) *Rate of radial advance.*

The rate of radial advance for the strain CJ and its saltants (N and 18 G media) is given in Table IX.

TABLE IX.

Strain.	Standard Medium. Radius in cm. day 7.	[1] Radial Advance per day.	Radius in cm. day 9.	Radial Advance per day (days 7–9).	18 G Medium. Radius in cm. day 7.	[1] Radial Advance per day.	Radius in cm. day 9.	Radial Advance per day (days 7–9).
CJ	4·20	0·70	4·50	—	4·10	0·69	4·50	—
CJ 1	4·10	0·69	4·50	—	3·90	0·65	4·50	—
CJ 1, 1	3·30	0·56	3·70	0·20	2·20	0·37	3·00	0·45
CJ 1, 1, 1	1·90	0·34	2·40	0·25	2·00	0·34	2·45	0·23
CJ 1, 1, 2	3·60	0·60	4·50	0·45	2·70	0·45	3·30	0·30
CJ 3	3·75	0·63	4·40	0·33	2·50	0·42	2·65	0·08
CJ 4	4·70	0·78	4·50	—	4·00	0·67	4·50	—

It will be seen from the table that CJ, CJ 1, and CJ 4, make vigorous growth, in fact the plates were almost covered on the seventh day. With CJ 1, CJ 1, 1, 1, and CJ 1, 1, 2, however, the plates were not covered on the ninth day. The strains CJ 1, 1, CJ 1, 1, 1, and CJ 1, 1, 2 show a certain amount of staling, thus with CJ 3 (N. medium) the rate for the first seven days is 0·63 cm. per day, during the next two days, however, the rate has fallen to 0·33 cm. per day.

The saltants may be provisionally classified as follows:

a. Spores of the *culmorum* type.

CJ — Mycelium well developed.
Sporodochia present.
Chlamydospores — development early, very numerous.
Substratum—strongly coloured.

[1] In calculating the rate an allowance of one day is made for spore germination.

Sporing scattered or sporodochia not prominent, substratal colouring feebly developed or absent.

- CJ 1 — Chlamydospores — development early, fairly numerous.
- CJ 1, 1 — Chlamydospores — development after eight days, exceptionally numerous. Spores rarely observed.
- CJ 4 — Chlamydospores — development after eight days, relatively few. Spores not uniform.
- CJ 1, 1, 1 — Chlamydospores—development after eight days, exceptionally numerous. Spore development late.

b. Spores more or less fusoid.

Sporing scattered.

CJ 1, 1, 2 — Chlamydospores present. Substratal colouring feebly developed.

c. Spores of the *Elegans* type.

Mycelium feebly developed. Spore masses confluent.

CJ 3 — Chlamydospores present. Substratal colouring feebly developed, or absent.

IV. Inoculation of Apples with Certain Parent and Saltant Strains.

In October 1925, 110 Cox's Orange Pippin apples were inoculated with the eleven strains, SG, Sa, D, CJ, CC, CC 1, P, P′, Pp, L and T—ten apples for each strain, using the method of inoculation devised by Granger and Horne (2). The apples thus inoculated were wrapped in sterile grease-proof paper and sent to Cambridge, where they were stored under constant temperature (12° C) and hygrometric conditions. After eighty-five days storage the apples were returned to London for examination, and the amount of decay due to the activity of the strains was estimated. Each apple was first weighed, the rotted tissue was then very carefully removed, and the apple was weighed a second time. The difference between the two weights yielded the amount of rotted tissue in the apple. From these figures the percentage weight of tissue rotted by each strain was obtained.

Gregory and Horne (3) have recently shown that percentage weight does not afford a very satisfactory measure of the attacking power of a fungus, and that a much more accurate estimate can be made by calculating the rate at which the fungus invades the tissues (rate of radial advance). Accordingly the rate of radial advance has been found for each

strain (see 4, p. 446), and is given together with the percentage weights in Table X.

In order to compare the virulence of fungal strains with reference to the same variety of apple, the apples should be examined not later than the time when one sample of the series first shows a completely rotten apple (3, p. 439). In the present case twenty-seven apples had completely decayed, having been held in storage too long. For this reason the rates of radial advance for the strains SG, Sa, D, CJ and CC are actually higher than the calculated values given in the table.

TABLE X.

Rate of Invasion for Certain Strains.

Strain.	Mean Apple in grm.	Mean Radius in cm.	Radial Advance.	Percentage Wt. of Rotted Tissue.	Radial Advance in cm.	Rate of Radial Advance in cm. per day.	No. of Apples completely Rotted.
SG	50·9	2·48	1·33	96·4	3·298	0·0388	5
Sa	51·0	2·48	1·37	98·6	3·398	0·0400	8
D	49·4	2·44	1·14	75·6	2·782	0·0327	5
CJ	58·8	2·60	1·23	87·6	3·198	0·0376	6
CC	50·7	2·48	0·79	51·6	1·959	0·0230	3
CC 1	53·4	2·51	0·21	3·2	0·527	0·0062	0
P	47·4	2·41	0·62	22·7	1·494	0·0176	0
P′	51·8	2·49	0·31	9·2	0·772	0·0091	0
Pp	46·8	2·41	0·12	3·1	0·289	0·0034	0
L	57·0	—	0·00	0·0	0·000	0·0000	0
T	51·1	2·48	0·37	7·1	0·918	0·0108	0

It will be seen from the table that the strains of the *discolor* group (SG, Sa and D) do not differ from one another greatly in virulence. With regard to the *culmorum* group (CJ, CC and CC 1) the strain CJ corresponds very closely with SG, CC is less active, and CC 1 a saltant derived from CC, is least active, the rate of invasion per day for CC being nearly four times that shown by the saltant. The three strains of *F. polymorphum* are less active than CC: the rate for P is nearly twice that of P′, and five times that of Pp.

Since the apples used for inoculation with the Discolor strains were obtained from the same source as those employed for the work on *Fusarium* described by Horne and Gregory (4), the activities of the two sets of strains have been compared with the result given in Table XI.

It is evident from the table that the first four Discolor strains given in column 3 are much more active than the most active *Fusarium* strain (D) given in column 1. The strain CC falls into Horne and Gregory's first group comprising D, B 1, C 1 and C 2, 1, which do not differ significantly (4, p. 458), P, T and P′ fall into the intermediate group which comprises A and D 2, and the saltant CC 1 into the group of weak saltants (B 1, 1 and B 1, 1, 1)

derived from B 1. The strain Pp is the least active of the Discolor strains with a rate of radial advance only one half that of B 1, 1 and B 1, 1, 1. It is interesting to note that the three weak saltants B 1, 1, B 1, 1, 1, and CC 1 are of the pionnotal type.

TABLE XI.

Comparison of the Activities of Two Groups of Fusarium Strains (Cox's Orange Pippin, 1925–6. 12° C.)

Fusarium Strains. H and G (4).		Fusarium Strains. Section Discolor.	
Strain.	Radial Advance in cm. per day.	Strain.	Radial Advance in cm. per day.
—	—	Sa	0·0400
—	—	SG	0·0388
—	—	CJ	0·0376
—	—	D	0·0327
D	0·0230	CC	0·0230
C1	0·0220	—	—
C2, 1	0·0200	—	—
A	0·0150	P	0·0176
C3	0·0140	—	—
D2	0·0098	T	0·0108
		P′	0·0091
B1, 1	0·0066	CC1	0·0062
B1, 1, 1	0·0067	Pp	0·0034
—	—		

V. GENERAL DISCUSSION.

The observations given above show that in several instances the differences existing between the saltants derived from a given parent strain are greater than those which separate one variety from another of the same species, and even one species from another.

The three original strains of *F. culmorum*, CC, CJ and CB, used in this investigation, although exhibiting certain general morphological differences, nevertheless agree in spore type. The saltants derived from the original *culmorum* strains, however, formed spores which did not always conform to the *culmorum* type. The average *culmorum* spore is slightly bent, it attains its greatest width either in the middle, or more frequently beyond the middle segment, and has abruptly terminated ends, and the septa are closely set. The saltant CJ 3, however, produces a long symmetrical spore with gradually attenuated ends entirely unlike the average spore of *F. culmorum*, and indeed, on the criterion of spore-shape, the saltant would actually be excluded from the section Discolor to which its parent belongs. The spore of CJ 3 resembles far more closely those figured by Sherbakoff for certain species of the section Elegans. Again, CJ 1, 1, 2 has distinctly fusoid spores. Finally the saltant CC 1, which differs strikingly from its parent strain (CC)

in sporing habit, also differs from it in producing spores which are usually bent at the apical end. The same 'bent' type of spore is also characteristic of CJ 1. Thus altogether four different types of spore are shown by the saltant strains of *F. culmorum*: (*a*) the *culmorum* type (CJ, CJ 4); (*b*) the *culmorum* 'bent' type (CJ 1); (*c*) the fusoid type (CJ 1, 1, 2); and (*d*) the Elegans type (CJ 3): three of these types (*b*, *c* and *d*) exhibit greater differences than are shown by any two typical Discolor species examined during this investigation. The species *F. clavatum* and *F. subpallidum* were not studied by the writer, but a reference to Sherbakoff's description of them (6, p. 230 and 234) will show that the differences separating these two species are far less than those existing between saltant and saltant. The same argument holds for the strains SG, SB, Sa and D (*discolor* group). Although the spores of individual strains were not distinctive, those of the saltants differed with regard to uniformity in shape and length, and the degree of septation. Thus SG 1 and SG 4 showed fairly uniform spores, but with SG 2 and SG 3 the spores lacked uniformity, and a certain percentage of short spores was present (including spores of the arthrosporal type).

Some of the criteria which have been used to distinguish one species from another will now be considered. The main distinction between *F. sambucinum* and *F. sulphureum* evidently rests on the character of substratal colouring. Colour is present in the former and absent in the latter. It is obvious from the results obtained in this investigation that this character alone cannot serve to distinguish species from species. Thus the strain CJ produces strong substratal colouring, but the saltants CJ 1 and CJ 3 develop feeble colouration. Again, substratal colouring is present in the case of *F. polymorphum*, but with *F. polymorphum* var. *pallens* it is absent.

It is difficult indeed to find any sound criterion for distinguishing between *F. sambucinum* and *F. sulphureum*. It is also difficult to distinguish between these species and *F. culmorum* and *F. polymorphum*, since the spores of *F. polymorphum* do not differ to any marked extent (in the same media) from those of *F. sulphureum* and *F. sambucinum*; again, the spores of *F. culmorum*, in certain media, resemble very closely those of *F. sulphureum*. Other characters also fail in attempting to delimitate the species.

According to Sherbakoff, the section Discolor is distinguished from the neighbouring sections Elegans and Martiella by the following characters:

(1) The spores are typically somewhat attenuated.
(2) The nature of the substratal colouring.
(3) The absence of terminal chlamydospores.
(4) The absence of microconidia

The shape of the spore has been discussed already in some detail. The spores produced by the saltant CJ 3 (section Discolor) are distinctly of the Elegans type.

With regard to substratal colouring, Sherbakoff states that vinaceous, drab grey, tawny olive, and blue colours are absent in the section Discolor. With the Discolor strains studied vinaceous colours were found to be of fairly frequent occurrence, while tawny olive was present in the substratum in the case of at least one of the strains.

Sherbakoff's statement that terminal chlamydospores are absent in the section Discolor is not in accordance with the results obtained in the present investigation. Chlamydospores, both in terminal and intercalary chains, are of fairly common occurrence. It should be stated that no strains with the chlamydospore-forming character absent have been derived from a chlamydospore-producing parent. Nevertheless, differences occur in the case of saltants derived from such a parent, related to the time taken for chlamydospore development and the number of chlamydospores produced.

With regard to the question of microconidia (short spores of low septation) it has been shown that with certain authentic strains they are present and with others (CJ) absent. In the former case the percentage of such spores produced varies with the medium used. Thus the strain SB on the 8 G medium produced only microconidia (average septation 1·10). It has been shown that saltants derived from a given parent vary considerably in their capacity for producing short spores (SG saltants), and in this connexion it is interesting to note that Sherbakoff has described a variety of *F. arthrosporioides*—*F. arthrosporioides* var. *asporotrichius*—which differs from the type in the typical absence of sporotrichial sporodochia.

F. lolii and *F. trichothecioides* are mainly characterized by their capacity to form microconidia, but in the light of the facts just reviewed it is difficult to determine whether this character really serves to delimit these two species from the other forms considered here.

With regard to the strain *F. discolor* var. *triseptatum* some doubt exists as to whether the fungus is *F. discolor*, or whether indeed it actually belongs to the section Discolor, since numerous pear-shaped spores resembling those formed by *F. sporotrichioides* were present in all the media used.

In conclusion, the Discolor strains show certain points of contact with other sections of the genus—for example, the saltant SG 3 derived from *F. sulphureum* in the occurrence of the arthrosporial type of spore, with the section Arthrosporiella: the strain CJ of *F. culmorum* in the nature of its chlamydospores, with the section Ferruginosum: and the saltant CJ 3 derived from *F. culmorum* in its spore characters, with the section Elegans. Divergences of this kind show the need for reviewing the validity of the so-called species of *Fusarium* in the light of far more comprehensive studies in saltation in the genus than the present writer has been able to undertake.

SUMMARY.

1. A comparative account of certain strains of *Fusarium* (section Discolor) is given, considering chiefly the rate of radial advance, mycelial development, substratal colouring, method of sporing, and the shape and septation of the spores.

2. During the course of the comparative work several saltants were obtained from strains of *F. sulphureum*, *F. polymorphum*, and *F. culmorum*. The mode of origin of the saltants and their salient morphological characters are described.

3. As a result of studying the available Discolor strains and saltants in detail, it was found that the specific characters, in so far as the Discolor species are concerned, are not distinctive, since the saltants derived from a given parent may show a greater divergence in critical characters than that evident between species and species.

4. The criteria used hitherto for the sectional grouping of the recognized species, for example, spore shape, nature of the substratal colouring, chlamydospores and their disposition, presence or absence of short spores (microconidia), &c., are found to be entirely unsatisfactory, since saltants derived from a given parent may show spores of more than one sectional type; for example CJ 3 (Elegans type), and CJ 4 (Discolor type) derived from *F. culmorum*, and the same holds good for the saltants when other characters are considered.

5. Ten of the Discolor strains and saltants were found to be capable of causing decay in the Cox's Orange Pippin variety of apple. The strains differed greatly in their attacking power, and the saltants tested exhibited less virulence than their respective parent strains.

This work was carried out under the supervision of Dr. A. S. Horne, to whom the author is greatly indebted for valuable help and criticism. The author's thanks are also due to Professor V. H. Blackman for the great interest he has taken in the investigation.

EXPLANATION OF PLATES IX AND X.

Illustrating Dr. J. H. Mitter's paper on *Studies in the Genus Fusarium. VII.* Saltation in the Section Discolor.

PLATE IX.

Figs 1–4. *F. polymorphum.* Changes in the sporing throughout the Asparagin series. (0·2A–2A) as shown by the strain P.

Fig. 1. 0·2A. Sporodochia small, dark, vinaceous.

Fig. 2. 0·5A. Sporodochia at (*a*) few, small, (*b*) pale buff pink, (*c*) small, scattered, dark vinaceous.

Fig. 3. 1A. Sporodochia as in Fig. 2, but much more numerous.

Fig. 4. 2A. Sporodochia larger, cinnamon pink.

Fig. 5. *F. polymorphum*, strain P, showing marginal zone of blue sporodochia (*b*).

PLATE X.

Fig. 6. *F. sulphureum*. Sectoring culture showing the saltants SG 2 and SG 3.

Fig. 7. *F. sulphureum*. Sectoring culture showing the saltants SG 4 and sector consisting of SG 1 and SG 3.

Fig. 8. *F. sulphureum*, showing sclerotia (strain SG).

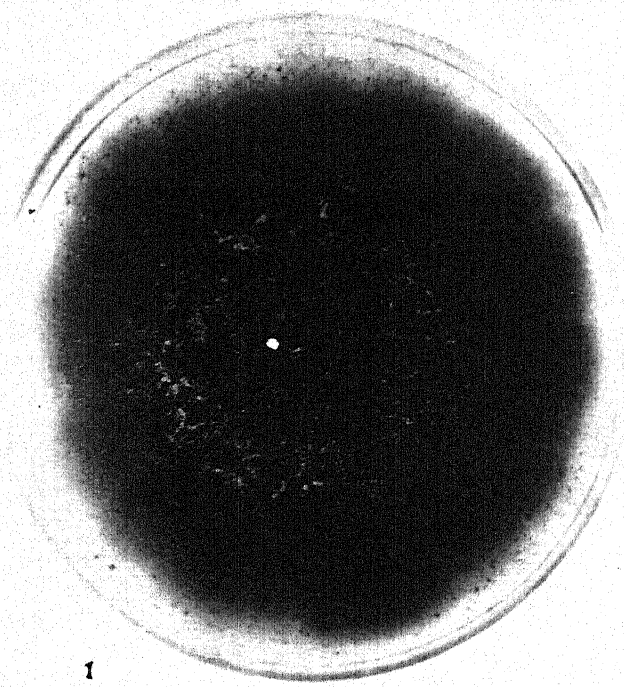
Fig. 9. *F. culmorum*. Sectoring culture viewed from below, showing the slow-growing saltant CJ 2 (dark centre) and fast-growing parent strain CJ.

Fig. 10. *F. culmorum*. Sectoring culture showing the pionnotal saltant CJ 3 (smooth, white, central portion) and the parent strain CJ.

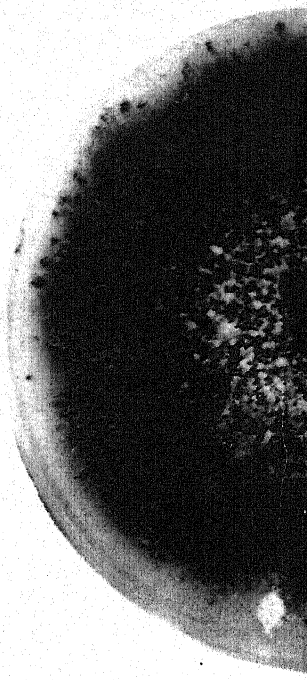
Fig. 11. *F. culmorum*. Sectoring culture showing the saltant CJ 4 (light portion) and parent strain, CJ (dark portion).

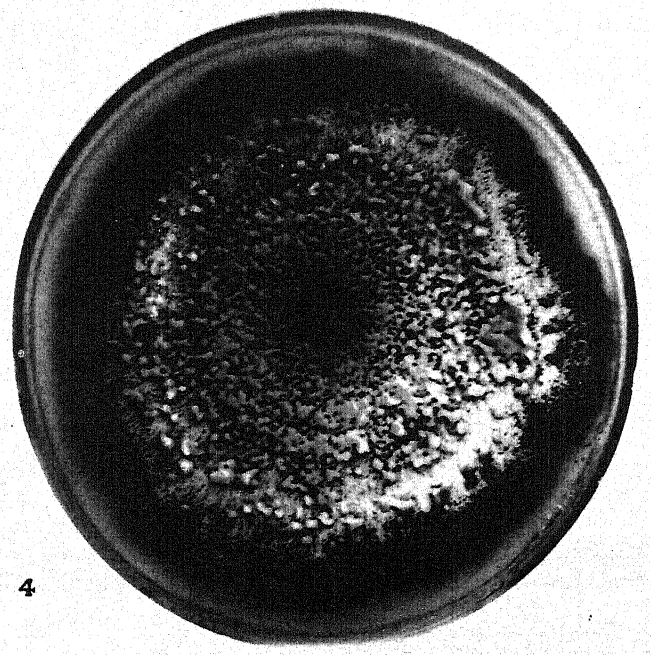
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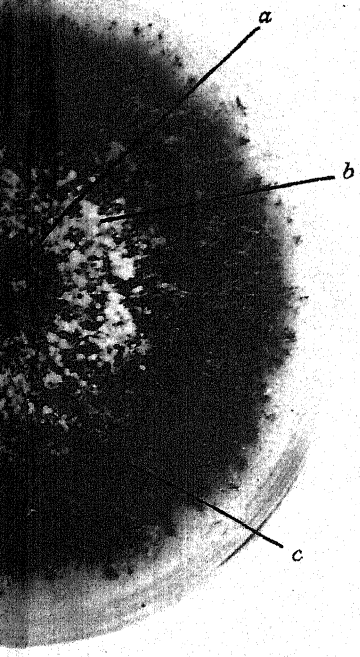
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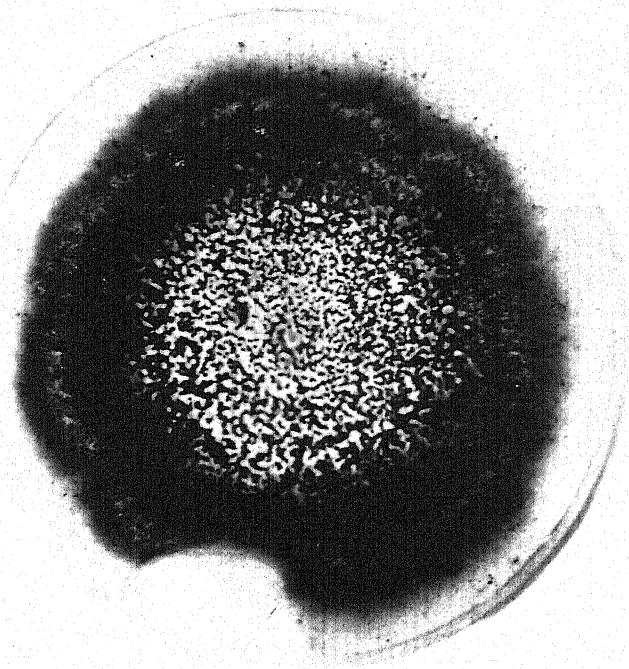


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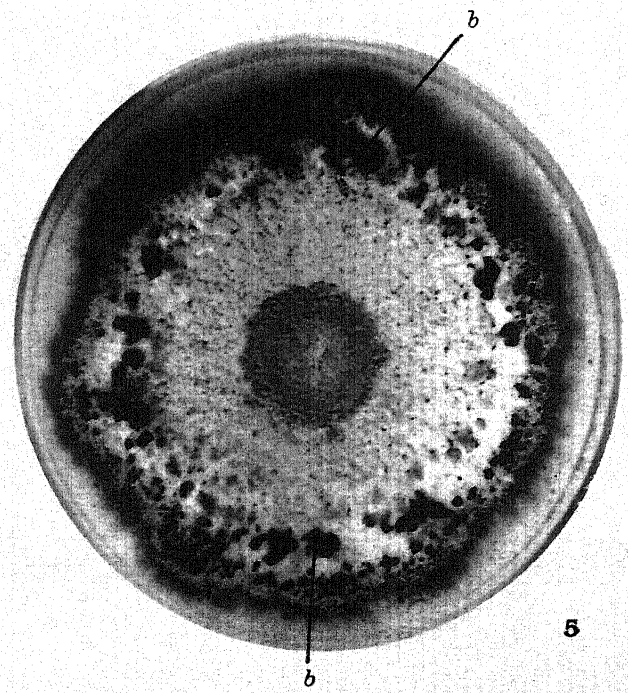
MITTER — SALTATION IN FUSARIUM.



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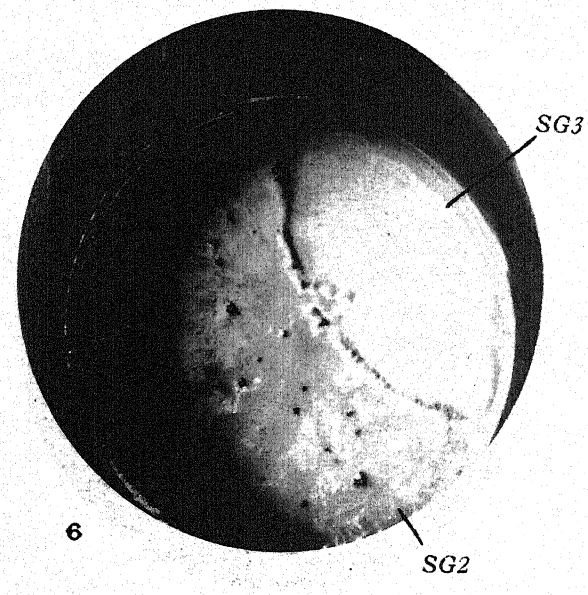


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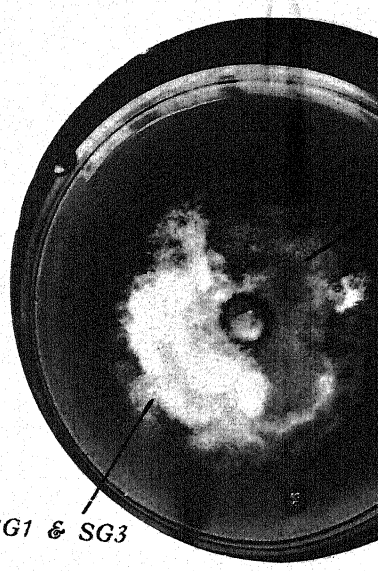


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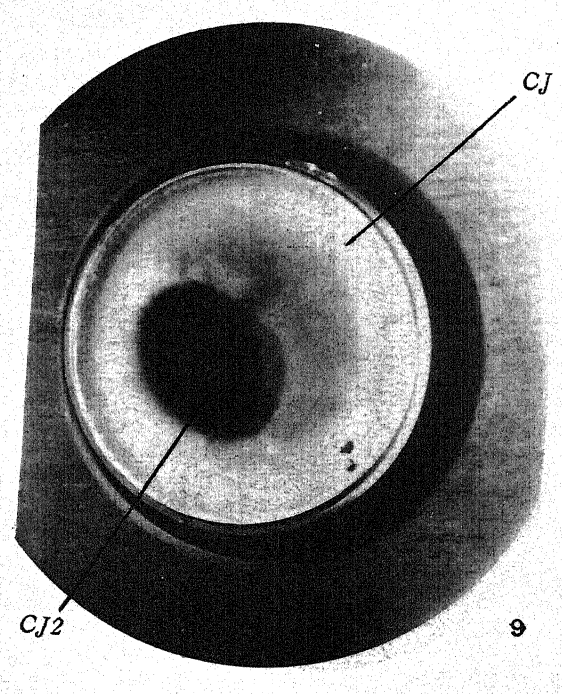
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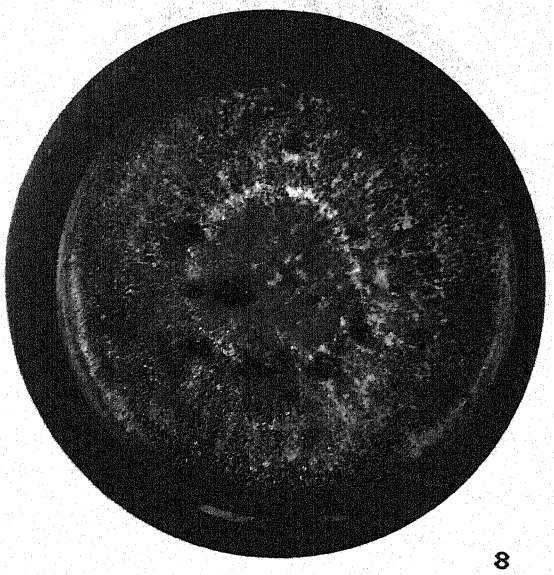
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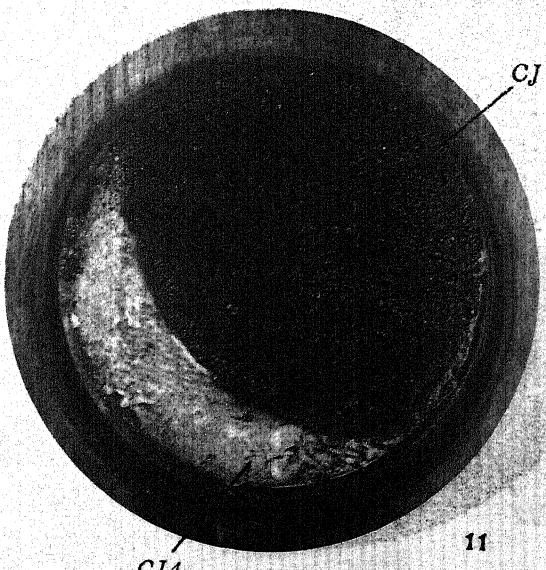
MITTER—SALTATION IN FUSARIUM.





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NOTES.

THE METHOD OF SPORE DISPERSAL OF THREE SOUTH AFRICAN SPECIES OF ISOETES.—During the last few years three new species of *Isoetes* (shortly to be described in Trans. Roy. Soc. of South Africa, vol. xvii) have been found in the south-western part of the Cape Province. One of these, *Isoetes capensis*, first collected by Miss E. Stephens of the Cape Town University, occurs in abundance in several shallow, temporary vleis in the Cape Peninsula, and is also known from marshy ground on the Stellenbosch Flats and from a water furrow in the neighbourhood of Somerset Strand. The other two, *I. Stephansenii* and *I. stellenbossiensis*, have up to the present been found only on the Stellenbosch Flats, where they often grow together in soil which is waterlogged for at least a part of the rainy season. *I. stellenbossiensis*, however, differs from the other two species in its xerophytic characters, and in its ability to occupy drier areas. All three species disappear completely, except for the corms, during the dry summer, and reappear soon after the beginning of the rainy season. The growing apex of the dormant corm is protected by overlapping scale leaves.

The presence of abundant material of *Isoetes* within easy distance of Stellenbosch suggested an investigation into the method of spore dispersal. Observations in the field were made at frequent intervals throughout the year, and were supplemented by the study of material kept in the laboratory and plant house.

The conditions under which the three Stellenbosch species grew are very similar to those described by Osborn (this Journal, xxxvi, 1922) for *I. Drummondii*, but the manner of spore distribution has proved to be entirely different.

By the end of November or early in December most of the green leaves have withered, and before long all traces of the laminae have disappeared completely. The leaf-bases, with the sporangia in their axils, remain attached to the corms which are usually buried in the soil, sometimes to a depth of five or six centimetres. There is no trace in any one of the species studied of the formation of mucilaginous tissue at the bases of the sporophylls, nor are the sporangia elevated to the surface of the soil. The walls of the sporangia decay *in situ*, leaving heaps of naked mega- and microspores among the old corm scales. By the end of the year very few of the spores of the preceding season can be found about the corms, and this fact, together with the presence of young sporophytes at some distance from the older plants, suggested an effective method of spore dispersal.

Early in September, 1928, after several days of heavy rain, a visit was paid to a large seasonal swamp on the Stellenbosch Flats where *Isoetes* was known to occur. Both *I. stellenbossiensis* and *I. Stephansenii* were found in abundance, the former extending to the drier parts and reaching the heath zone. On digging up some of the plants large numbers of earthworms were observed in the moist soil surrounding

the corms. The earthworms belonged to several distinct species, but it has not as yet been possible to get them identified. Several of these earthworms were removed, carefully washed, and placed in a covered jar together with pulped filter paper. After three days the castings were examined, and were found to contain an abundance of mega- and microspores belonging to both species of *Isoetes*. A few of the megaspores had broken along the commissural ridges and the equator, but the majority were entire. On dissection, both megaspores and microspores were found to have passed through the body of the worm without injury. Unfortunately the parts of the Flats where *I. capensis* occurs were only visited a fortnight later, and by this time the heaps of spores about the corms had been largely dispersed; but the presence of earthworms in the damp soil suggested that in this species also they played a similar role. A few of the earthworms were removed as before, and several microspores and one megaspore were recovered from the castings.

It is probable that earthworms are largely responsible for the distribution of the spores of *I. capensis* on the Cape Peninsula, though the occurrence at certain times of the year of numbers of small, fresh-water snails (identified by Dr. A. Porter as *Temisia ventricosa*) in the mud in which the plants are rooted suggested that they, too, might be concerned in the process. As it was not possible to make a special journey to the Cape Flats to investigate this point in the field, several of the snails were kept for a few days in a vessel containing *Isoetes* and then removed to a dish of fresh water. The excreta, on examination, were found to contain a certain number of microspores but no megaspores. Some displacement of the megaspores may, however, be caused mechanically by the movement of the snails among the corms. The possibility of tadpoles acting as agents for spore dispersal in the case of *Isoetes* plants growing in marshy ground was also investigated. The tadpoles used for the experiment were those of the common *Rana grayi*, but the results showed them to be of little or no value in effecting spore distribution.

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A SATISFACTORY METHOD OF STAINING FUNGAL MYCELIUM IN WOOD SECTIONS.—Great difficulty has been encountered in finding a suitable stain for clearly differentiating fungal mycelium in wood. In the case of many of these fungi possessing uncoloured mycelium, it is impossible to trace the hyphae through the wood elements without the use of some stain; indeed, in many cases where the hyphae are of fine diameter, and in areas of incipient decay, where few if any bore-holes are present, or in those fungi which utilize the pits only, the fungus mycelium cannot be seen without the use of some stain.

Such stains as cotton blue with lactic acid, Bismarck brown, and methyl violet afford fair differentiation in certain cases, but all the stains which have been tried appear uncertain in their results, and many are both laborious in practice and soon fade, e. g. Pianeze III b.

As it is well known that safranin stains the lignified walls, and picro-aniline blue has been used for the staining of mycelial wefts, it was thought that possibly a combination of these two might prove satisfactory as a double stain.

Results were found to be satisfactory, the procedure adopted being as follows:

1. Stain section in 1 per cent. aqueous safranin. (It will be found sufficient, in most cases, to cover the section with the stain and to drain off immediately).

2. Wash off all superfluous stain in water, leaving the section slightly over-stained.

3. Stain in picro-aniline blue, made up as follows:

To 25 c.c. of saturated aqueous aniline blue add 100 c.c. of saturated aqueous picric acid.

Cover the section with the stain and warm over a flame until just on the point of simmering.

4. Wash out with water all the blue, which comes out easily.

5. Wash in changes of spirit, and finally in absolute alcohol.

6. Clear in clove oil, and complete in the usual way, finally mounting in Canada balsam.

The resulting preparation should show the lignified walls stained red and the fungus mycelium a clear blue.

If the wood is badly decayed in parts, these areas will take up a certain amount of blue stain, but the mycelium remains clearly differentiated. This stain has been tested over a long period on all types of wood-destroying fungi, and also on other wood-inhabiting fungi (Basidiomycetes, Ascomycetes, Fungi Imperfecti, and Moulds) and has been found satisfactory in every case.

A few of the fungi of frequent occurrence on which the stain has been tested include the following:

Basidiomycetes.

Stereum hirsutum.
„ *sanguinolentum.*
„ *purpureum.*
Corticium, spp.
Peniophora, spp.
Merulius lacrymans.
Coniophora cerebella.
Poria vaporaria.
Polyporus versicolor.
„ *hispidus.*
Trametes pini.
Daedalea quercina.
Fomes annosus.
„ *ferruginosus.*
„ *fraxineus.*
Irpex, spp.
Pholiota adiposa.
Pleurotus ostreatus.

Ascomycetes.

Ceratostomella pini.
„ *piceae.*
Daldinia concentrica.
Nectria, spp.

Fungi Imperfecti and 'Moulds'.

Trichoderma lignorum.
Penicillium, spp.
Aspergillus, spp.
Mucor, spp.

The ease and quickness of making permanent mounts, combined with the fact that the progress of the finest hyphae can be traced accurately throughout the wood elements, should commend it to the forest and timber mycologist and to all who make a study of decay in wood.

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ON THE CUTICLE OF CERTAIN SPECIES OF NEUROPTERIS, BRONGN.—In 1914 Miss Wills (Geol. Mag., vii, vol. i, pp. 385–90) wrote a brief account of the structure of the cuticle of *Neuropteris heterophylla*, Brongn., in which she described and figured stomata and water-pores. She also noted the occurrence of papillate hairs on one type of pinnule, and mentioned the similarity of epidermal characters existing between the pinnules examined and certain closely related *Cyclopteris* pinnules.

The following notes are the outcome of further research on *Neuropteris* pinnules, using Walton's and Ashby's methods of transfer preparation. It was found that if the pinnule was of a blackish colour and of a solid appearance Walton's method gave the best result; if, on the other hand, the specimen was brownish and gave indications of the preservation of tissue Ashby's method was preferable. In a few cases where the pinnule was well preserved in soft shale, the following method was used: the specimen was soaked in water until it was possible to remove pieces of the lamina. These were macerated in Schulze's solution, washed in water, cleared in oil of cloves or terpinol, and mounted in Canada balsam.

The specimens examined were those belonging to the *Neuropteris flexuosa* group (Bolton, Journ. Linn. Soc., vol. xlvii, pp. 295–326, 1926), i. e. *N. flexuosa*, Sternb., *N. gigantea*, Sternb., *N. tenuifolia*, Schl., *N. macrophylla*, Brongn., also *N. rarinervis*, Bunbury, and *N. obliqua*, Brongn. All the specimens examined were found to possess hairs on both surfaces of the lamina, the greater number being found on the lower surface except in *N. scheuchzeri*, Hoffm. These hairs occur sparingly on the veins, but in greater numbers on the areas between the veins. They are also more numerous near the base of the pinnule, and become comparatively few in number near the margin, which in most cases is curved under. As far as could be ascertained there do not appear to be hairs present underneath this curved portion.

Each hair rises from a broad base and tapers to a blunt apex. It is unbranched and unicellular with moderately thick walls. Few hairs are found complete, most being broken off near the base; this renders the hairs very difficult to recognize under the microscope, and with a hand-lens they cannot be seen. No essential difference was found in the characters of the hairs attached to the pinnules of the various species of *Neuropteris* examined.

Miss Wills remarks upon 'the presence of small papillate hairs on one surface of the leaf in type β'. In a specimen of *N. gigantea*, Brongn., examined, there were, on the lower surface, what appeared to be small papillate hairs, but closer examination with different lighting showed that these supposed papillate hairs were really the bases of hairs of the ordinary type, the upper portion being missing. I venture to suggest that the papillate hairs recorded by Miss Wills are similar. The structure and position of the hairs on these *Neuropteris* pinnules are very similar to those described and figured by Dr. Florin (Arkiv. f. Bot., B. xx*a*, no. 13, pp. 1–19, 1926) as occurring on certain species of *Cyclopteris*.

The character of the stomata agreed in all points with those described and figured by Miss Wills. In one or two of the preparations small pores were discovered near the margin of the pinnule in the region of the ends of the veins, similar to those described and figured by Miss Wills as water-pores. There is a close similarity

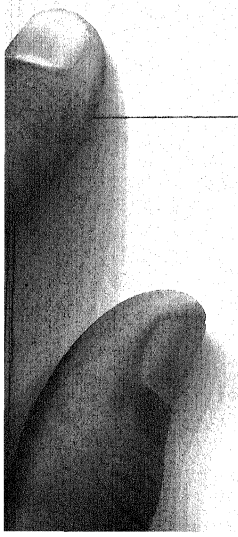
in structure between the hairs and stomata of *Neuropteris*, spp., and *Cyclopteris Felixii* (see Florin, Pl. III, Fig. 2).

Owing to the great difficulty in obtaining suitable preparations of cuticle, it is not at present possible to identify the various species of *Neuropteris* by their cuticular structure.

In conclusion, I should like to thank Mr. J. Walton for his kindness in showing me this method of removing fossil plants from the matrix, and for material I am also indebted to Mr. Walton and to the Keeper of the Geological Department, British Museum (Nat. Hist.), to Dr. A. E. Trueman, and to the Director, Bristol Museum.

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Studies in the Genera Cytosporina, Phomopsis, and Diaporthe.

I. On the Occurrence of an 'Ever-saltating' Strain in Diaporthe.

BY

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AND

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With seven Figures in the Text.

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I. INTRODUCTION.

DIAPORTHE PERNICIOSA was isolated on several occasions from spots which developed naturally on Bramley's Seedling apples during the autumn of 1927. From one of these isolations several subcultures of monohyphal origin were prepared, all of which resembled each other, and were termed DH_B. Eventually from one of these subcultures a strain (DH_C) was obtained which differed from the parent markedly, as will be evident

from the photograph represented in Fig. 1. These two strains, parent and saltant, are compared below:

DH_B—White. *Copious, silky, erect, aerial mycelium disposed in one or more zones.* Infertile black perithecia developing late. Substratum brownish, darkening with age. *Fast growing.*

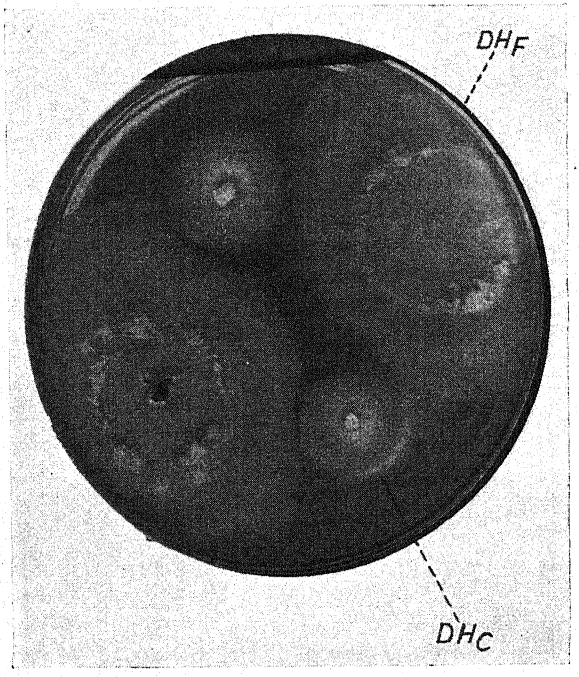


FIG. 1. Photograph of 12 days old plate culture, showing the strains DH_F and DH_C side by side.

DH_C—White. *Surface of culture smooth. Aerial mycelium entirely absent.* Infertile black perithecia developing late. Substratum brown, darkening with age. *Slow growing.*

It is the strain DH_C with which we are mainly concerned in this paper. It exhibits saltation of a new type, since the parent strain becomes completely transformed into the new strain.

Unlike most of the saltating strains described by various investigators, DH_C shows no sectorial differentiation. The growth is perfectly homogeneous and its character does not change with time in any given generation. It was not until several subcultures had been made, using mycelium for the inocula taken from different parts of a culture that any difference could be detected behind this apparent uniformity. Then it was found that the growths originating from mycelium taken from the older parts of the culture

differed completely from those originating from mycelium taken from the younger parts, which bred true to the parent; thus it was evident that the older mycelium had changed or saltated, although no expression of saltation was detectable in the body of the parent culture.

It was considered eminently desirable to follow the question farther and to attempt to analyse the factors concerned. It was thought that the observed change in the strain might have been due to any one or a combination of the following factors: (1) age of the mycelium, (2) nutritive conditions, (3) temperature. Accordingly, an investigation was carried out in considerable detail, in different media, including media varying in their acid and alkaline constituents, and so on. This investigation resulted not only in establishing that saltation takes place at a definite stage of development, but also in establishing the remarkable fact that a definite relation holds between the time and place at which the saltation becomes manifest and the rate of spread of the culture. It was further found that the saltation is cyclic.

The following experimental method was adopted throughout the investigation. A plate was inoculated in the centre, and when the culture had made sufficient growth, fragments of mycelium were taken as inocula from different points along given radial lines, starting very near the centre of the culture and working towards the edge, the position of each point being recorded. The inocula were transferred to plates, which were numbered according to the relative positions the former (inocula) occupied in the parent culture. The type of growth arising from each inoculum was noted. After a given interval of time, inocula were again taken from the recorded positions and serially transferred into plates, and ultimately a second series of growth forms was obtained. The growth forms arising on any two occasions were compared, and their relations with positions that furnished the inocula were noted. In order to eliminate as far as possible errors due to variations in the medium, inocula taken from both young and old parts of a given culture were transferred to different parts of the same plate (Fig. 1).

The following standard synthetic medium (3) was used throughout the experiments, except where stated otherwise:

Glucose	2 grm.
Asparagin	2 grm.
$MgSO_4$	0·75 grm.
KH_2PO_4	1·25 grm.
Starch (Potato)	10 grm.
Agar	15 grm.
Water	1 litre.

II. Relation of Saltation to Time.

A. *General Observations.*

The cultures of monohyphal origin, to which reference has already been made, were prepared from the parent DH_B, and it was a subsequent generation from one of these cultures which gave rise to DH_C, a descendant of

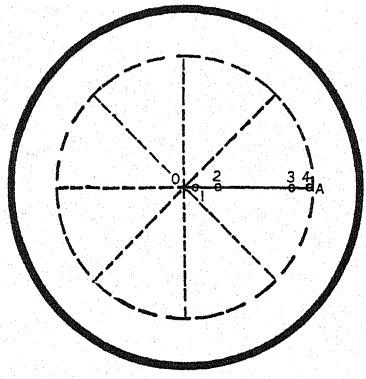


Fig. 2. Illustrating one of the radial lines along which inocula (1, 2) from the older part and (3, 4) from the younger part were taken from a plate culture of DH_C. Dotted lines denote other similar radial lines. Discontinuous circle denotes the growing edge of the culture. Continuous circle denotes the edge of the plate.

which (DH_{C-1}) was then chosen for special investigation. After DH_{C-1} had been grown at room temperature for two weeks, four inocula were taken along a given radial line, two (Fig. 2., 1, 2) from an older part, and two (Fig. 2., 3, 4) from a younger part of the culture. It was found that whereas the growths originating from inocula (1) and (2) resembled DH_B, those from (3) and (4) resembled DH_C (DH_{C-2}, e. g. DH_C of second generation). Now it was from a culture of DH_B that DH_C originated. Here the reverse was found, namely DH_B originating from DH_C. Although these cultures of DH_B, the original parent and saltant, closely resembled one another morphologically, it was deemed advisable to consider them separately, and accordingly the saltant DH_B was named DH_F.

The strain DH_{C-2} on subculturing behaved in the same way as the parent DH_{C-1}, that is to say, inocula taken from relatively old mycelium produced DH_{F-1}, and those from younger mycelium produced DH_{C-3}. Successive generations of DH_C behaved in a similar manner, as shown in Fig. 3, indicating that this saltation in the mycelium is inherent in the strain DH_C.

For the sake of convenience the region of the culture producing DH_C is named the DH_C-zone, and when part of this culture is so altered as to give rise to the DH_F strain, it is termed the DH_F-zone.

The saltant strain DH_F was then subjected to experiments on similar lines. It was seen that DH_F once obtained proved highly constant. Although repeatedly subcultured it did not show any change except on a solitary occasion when, in malt agar, it showed a sector resembling DH_C which was subsequently isolated. This saltation will be discussed later (p. 430).

B. *Detailed Observations.*

The relation between the age of the mycelium and the type of culture produced suggested that saltation took place at a definite time. Hence in order to find out the exact time when it occurred, some very detailed experiments were carried out (room temperature).

In this case, instead of removing a few isolated fragments of mycelium, successive fragments were taken starting from the centre of the culture, and working outwards so that a narrow strip of mycelium was entirely removed. The operation was repeated at regular intervals of time, always using a strip adjoining that removed on previous occasions. On each successive occasion the strip was longer than that previously taken, owing to the increment in the radius due to the growth of the culture. The method adopted is illustrated in Fig. 4, where O–A shows the strip taken on the third day, O–B that taken on the fifth day, O–C that taken on the sixth day, and so on. An effort was made to ensure that each mycelial inoculum was equal to 1 mm. of the radius, so that the total number of inocula taken would as a rule correspond with the total radius of the culture at that time. The inocula were on each occasion transferred in serial order to fresh medium in order to test their behaviour. The type of growth developing from each inoculum indicated the nature of the mycelium at the corresponding place in the parent culture at the time when the inoculum was taken. It was thus possible to compare the type of growth obtained on earlier and later occasions respectively from any given point in a culture, and ascertain thereby whether any change had been initiated or not. It must be stated here that one entire set of experiments was conducted from one definite plate culture of DH_C only. The experiment was begun with a culture three days old, and was pursued up to the time when it had attained the age of thirty-one days, working upon it periodically. The results are given in Table I.

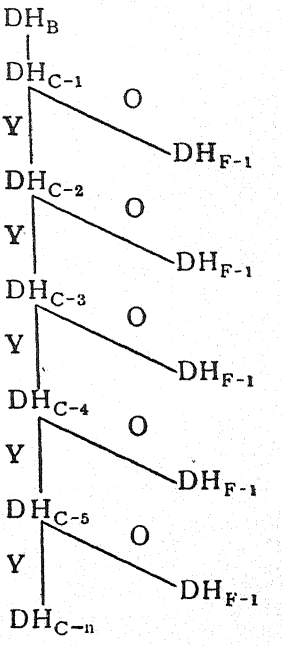


FIG. 3. Diagrammatic representation of the behaviour of DH_C in successive subcultures. O, old mycelium; Y, young mycelium.

TABLE I.

Radial Advance in mm.	Time in Days.	Type of strain originating from each successive inoculum.	Radius (mm.) C.	Radius (mm.) F.
	1			
	2			
3·0	3	C C C	3·0	0·0
	4			
5·0	5	C C C C C	5·0	0·0
6·5	6	F C C C C C C	6·0	0·5
7·5	7	F F C C C C C C	6·0	1·5
	8			
	9			
10·0	10	F F F F C C C C C C	6·0	4·0
	11			
	12			
	13			
13·0	14	F F F F F F F C C C C C C	6·0	7·0
	15			
	16			
	17			
18·0	18	F F F F F F F F F F F C C C C C C	6·0	12·0
	19			
	20			
20·0	21	F F F F F F F F F F F F F C C C C C C	6·0	14·0
	22			
	23			
	24			
	25			
	26			
	27			
	28			
	29			
	30			
30·0	31	F F F F F F F F F F F F F F F F F F F F F F F F F C C C C C C	6·0	24·0

C denotes DH_C strain.
F „ DH_F „

N.B. The nature of the mycelium present along any given radius of the culture is indicated by the letters C and F. Since each inoculum taken measures 1 mm. (approximately) the total number of C's and F's in a line gives the width in mm. of the DH_C- and DH_F-zones respectively.

The table shows the following points of interest:

(1) *Incidence of the Change on the 6th Day.* Up to the 5th day the culture always bred true, regardless of the positions from which the inocula had been taken. A change, however, was noticeable on the 6th day, when a mycelial zone ·5 mm. wide near the centre gave rise to DH_F (DH_F-zone), although DH_C had been previously obtained from the same place. The first demonstrable change had, therefore, taken place between the 5th and 6th day of growth. This result has been subsequently confirmed.

(2) *A Progressive Increase in Width of this Zone of Changed Mycelium (DH_F-zone).* The DH_F-zone had increased in width to 1·5 mm. on the 7th day, to 4 mm. on the 10th day, and so on.

(3) *The Zonal 'Advance' of the DH_F-zone.* In ordinary cases of saltation the change is initiated at a point near or at some distance from the centre, and shows as a well-marked conical sector. The tested mycelium in this case shows that the DH_F-zone 'advances' as a circular plate.

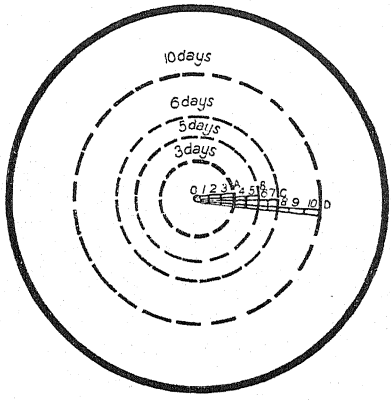


FIG. 4. Diagrammatic representation of experimental method adopted to note the incidence of saltation in time and place in a culture of DH_C. Successive discontinuous circles denote the advance of the culture at definite times in days. Nos. 1, 2, &c., denote successive inocula taken along radial lines.

(4) *The Extraordinary Regularity of the Change.* The radius of the culture on the 6th day is 6·5 mm., of which the first millimetre, starting from the centre, has been transformed into DH_F, the remainder 5·5 mm. (practically 6 mm.) remaining as DH_C. On the following day when the culture has made a further radial advance of 1 mm., a second millimetre directly adjoining the first has been transformed into DH_F. This goes on for each millimetre advance of the culture one millimetre of the oldest mycelium of DH_C is transformed into DH_F, so that DH_C is always limited to a zone of 6 mm. from the advancing edge of the culture, until the end of the plate is reached. Then with the cessation of the advance of the culture, the growth of DH_C stops, but the transformation of its oldest mycelium into DH_F continues at the former rate; so that eventually the whole DH_C is transformed into DH_F.

(5) The last point of interest is the sharp demarcation between the two. This is evident from the fact that inocula taken 1 mm. apart at the boundary give rise to DH_F and DH_C respectively whereas 1 mm. farther both give DH_C.

III. RELATION OF SALTATION TO NUTRITIVE CONDITIONS.

The characteristics of the DH_C and DH_F strains so far described are those as developed in the standard medium. To test the constancy of

these characters, and particularly to find out to what extent this age-phenomenon was subject to nutritive conditions, further investigations were carried on in several other media, namely: (1) Coons agar; (2) Richards agar; (3) malt agar. To each of these was added 1·0 per cent. of potato starch.

Growths in these media showed distinct departures from those observed in the standard medium, in three directions: (1) Irregularity in radial advance. (2) Rate of radial advance. (3) The growth characteristics.

Of these three deviations the last one is obviously the most important, since the recognition of the strains depends upon their growth characteristics. In spite of these deviations, however, DH_F was usually distinguishable from DH_C, especially in younger cultures, but in older ones some difficulties were experienced. DH_F always produced a thick mycelial network and also copious aerial mycelium, whereas in the case of DH_C, while young, the network was thin and the aerial mycelium absent, but as it grew older, copious aerial mycelium was produced from the advancing edge of the culture comparable to that shown by DH_F. It appeared as if DH_C had changed into DH_F at the margin. In order to determine this accurately, the behaviour of growths of DH_C of various ages was thoroughly investigated in Coons medium. The result showed the exclusive appearance of DH_C from mycelium taken from different parts of the younger cultures (5–6 days old) and tested on the standard medium. But similar tests using mycelium from older cultures revealed the fact that the first change appeared on the 7th day, and that from the 9th day onward the DH_F strain appeared both from the central region (the older part), as well as from the advancing edge of the culture (the youngest part). It was further observed that there was no definite order in the succession of DH_C and DH_F in the intermediate region of such cultures, when inocula were taken along a radial line. In old cultures (21 days) this irregularity ceased when the entire growth was converted into DH_F. The results are illustrated graphically in Table II.

Table II brings out the following interesting points:

(1) The presence exclusively of DH_C until the 6th day.

(2) The presence exclusively of DH_F in old cultures (21 days).

(3) The incidence of the change in older parts on the 7th day.

(4) The promiscuous blending of DH_C and DH_F, that is to say, the absence of any definite order in their succession in cultures more than nine days old.

(5) The appearance of DH_F at the growing edge, i.e. the youngest hyphae after the 9th day.

The first three results were consistent with those obtained with the cultures of the standard medium, and showed the same relation with age.

TABLE II.

(Coons medium.)

Time in Days.	Type of strain originating from each successive inoculum.	O
1		
2		
3		
4		
5	C C C C	C C C C
6	C C C C	C C C C C
7	C C C C F	F F C C C
8		
9	F F C C C F F F	F F F C C C
10		
11	F F F F C C F C F F	F F F C F C C C F F
12		
13		
14	F F F F F C F F C F F F	F F F F F C F C F C C F F F
15		
16		
17		
18		
19		
20		
21	F F F F F F F F F F F F	F F F F F F F F F F F F F F F
22		

O = Centre of culture—i. e., point of inoculation in a plate.
C = DH_C strain.
F = DF_F strain.

N.B. The records indicated by C and F in a line represent the types of growth obtained from inocula taken along a given diameter of the culture. The first inocula from opposite sides of the centre (O) were taken from very near the centre, the outermost ones from the growing edge; the other inocula were from intermediate positions, but not from any measured distance from the centre.

The last two results (4 and 5) were anomalous and needed explanation. It seems obvious that the blending of DH_C and DH_F is causally connected with the appearance of DH_F at the growing edge. The first explanation to suggest itself was, that the change had occurred at the growing edge, i. e. in the younger mycelium, but this seemed unlikely, since such a change in young mycelium might have been expected to appear at the very start of the culture. Furthermore, it will be seen from Table II that DH_F first made its appearance in the oldest part of the culture (DH_C) on the 7th day, while the younger parts were still like the parent. On the 9th day, this DH_F had 'advanced' a certain distance; it was then, and not till then, that DH_F was obtained from the growing edge too. This relation between the initiation of change in the older mycelium and the appearance of DH_F from the growing edge clearly indicated that the leading DH_F-mycelium had its origin from the central DH_F-zone, and that, growing side by side

with DH_C, it had eventually succeeded in outgrowing the latter, the result being the change from DH_C (in young cultures), to DH_F (in old cultures), through an irregular mixture of DH_C and DH_F in cultures of intermediate age. This supposition accounted well for the anomalous behaviour in Coons medium, and further showed that the difference between the two

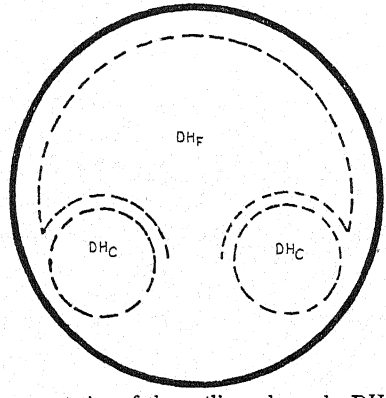


FIG. 5. Diagrammatic representation of the outlines shown by DH_C and DH_F when grown side by side in the standard medium.

media (Coons and standard) lay in this, that in the standard medium certain products of the preceding DH_C-zone prevented the growth of the DH_F mycelium which always lagged behind, whereas in Coons the absence or the neutralization of any such product allowed the DH_F mycelium to grow out.

This view found support in the fact that, when DH_C and DH_F were grown side by side in the standard medium, the former produced staling substances which inhibited the advance of DH_F when sufficiently close, whereas the staling substances produced by the latter (DH_F) appeared to have no effect upon the advancing mycelium of DH_C. The result was that DH_C always retained its full circular form, but DH_F, when sufficiently close to DH_C, assumed a crescented form (Fig. 5). This showed that DH_F was unable to grow in a medium staled by the strain DH_C. In order to confirm this, DH_C was grown in a plate of standard medium, the depth of the medium being about 1 cm. After three weeks, when it was thought that the staling products produced by the culture had diffused to the bottom, the medium was carefully turned upside down by means of a sterilized knife, and inoculated with young mycelial fragments of DH_F and older fragments of DH_C (i. e. potential DH_F-mycelium), at different places. The experiment was repeated several times. On every occasion inocula placed within the region corresponding to that occupied by DH_C on the other side failed to grow, although growths were obtained from those placed beyond the influence of the staling products.

It seemed obvious, therefore, that the initiation of change in the mycelial characters is independent of the medium, so long as it does not too greatly retard the growth of fungus, and that it is only the expression of this internal change which is conditioned by the nature of the medium.

The next line of inquiry followed was the elucidation of the factors which determine the behaviour of the changed mycelium. First of all the essential difference in the composition of synthetic media, such as Richards, Coons, and the standard medium were noted, which suggested that the change of behaviour with the medium might possibly be attributed to: (1) the nature of the available source of sugar, (2) the nature of the available source of nitrogen, (3) the concentration of the constituents, and (4) the acidity of the medium. Thereupon extensive cultural work was started on the standard medium, varying first the nature, then the ratio of the sugar and nitrogen constituents, and finally varying the concentration of some of the important principles of the medium. Certain irregularities in the growth form of DH_C could be detected when grown in some of these modified media. These irregularities, however, seemed to be correlated not with the nature of the constituents themselves, but with the altered acidity of the medium, mainly due to the utilization of some of the constituents by the fungus. This led to a special study of the last, namely, the acidity of the nutritive medium.

Since Coons agar medium is more acid than the standard, and since the relation between the DH_F and DH_C zones was indefinite in the former and definite in the latter, it seemed probable that by decreasing the acidity of Coons, and increasing that of the standard medium, a stage could be attained where the result would be reversed, that is to say, in the Coons medium the DH_F-mycelium will always follow the DH_C-mycelium, and in the standard medium the former will outgrow the latter. Hence the cultures were made in two different series:

(1) Cultures of DH_C in acid series using the standard medium (N).

(2) Cultures of DH_C in alkaline series using Coons medium with starch and replacing potassium nitrate with ammonium carbonate (C).

The following grades were employed:

$(N_A) = N + 0{\cdot}05\,\%$ malic acid.
$(N_B) = N + 0{\cdot}075\,\%$,,
$(N_C) = N + 0{\cdot}1\,\%$,,
$(N_D) = N + 0{\cdot}15\,\%$,,
$(N_E) = N + 0{\cdot}2\,\%$,,
$(N_F) = N + 0{\cdot}25\,\%$,,
$(N_G) = N + 0{\cdot}3\,\%$,,
$(N_H) = N + 0{\cdot}4\,\%$,,
$(N_I) = N + 0{\cdot}5\,\%$,,
$(N_J) = N + 1{\cdot}0\,\%$,,
$(N_K) = N + 2{\cdot}0\,\%$,,

$(C_A) = C + 0{\cdot}025\,\%\ (NH_4)_2CO_3$
$(C_B) = C + 0{\cdot}05\,\%$,,
$(C_C) = C + 0{\cdot}075\,\%$,,
$(C_D) = C + 0{\cdot}1\,\%$,,
$(C_E) = C + 0{\cdot}15\,\%$,,
$(C_F) = C + 0{\cdot}2\,\%$,,
$(C_G) = C + 0{\cdot}3\,\%$,,
$(C_H) = C + 0{\cdot}5\,\%$,,

The result obtained was true to expectation as summarized below:

Standard Medium (acidified). There was no change in the regularity of DH_C when grown in N_A. Definite irregularity in the succession of DH_F and DH_C, however, first made its appearance in (N_B). This irregularity increased with N_C and N_D, and culminated in N_E, N_F, and N_G, although no appreciable difference could be detected among the last three. At still higher concentrations of acid (N_H–N_K), owing to the inhibitory influence of the medium, the strain DH_C ceased to grow.

Coons (Alkaline). The irregular behaviour of DH_C in normal Coons medium was also observable in C_A–C_F, but a change to definite regularity was detected in C_G, both with regard to the growth of the culture and the succession of the DH_F and DH_C strains. This regularity was also evident in C_F, but to a smaller extent. With higher concentration of the alkali no further notable result was obtained.

Tables III and IV illustrate the change to irregularity and regularity in the succession of the DH_C and DH_F strains in the media N_E and C_F respectively.

TABLE III.

N_E = standard medium + 0·2 % malic acid.

Time in Days.	Type of strain originating from each successive inoculum. o
2	
4	
6	F C F | C C F
8	F C C F F | F C C F F
11	F F F C C F F F | F F C F C C C F F C C C
15	F F F F F F F F F F | F F F F F F F F F F F F F F

Explanations as in Table II.

A comparison between the Tables I and III and Tables II and IV will clearly show the effect of acidity and alkalinity on the succession of DH_F and DH_C strains. The extreme uniformity and regularity of this succession, so conspicuous a feature in the standard medium (Table I) has been entirely lost with the introduction of acid (Table III), so that in the younger growths the same measure of irregularity has been detected as found in Coons normal medium (Table II), and which ceases only when in older cultures, the whole growth has been converted into DH_F. On the other hand Table IV shows that the irregularity in succession of DH_C and DH_F as found in Coons medium (normal) had been completely altered into the regularity found in the standard medium by the introduction of the proper amount of alkali. Furthermore, in the last-named case the DH_F-zone always follows the DH_C-zone as in the normal standard medium.

These results prove conclusively that the dominance of one strain over another is purely a question of the concentration of the acid in the medium.

TABLE IV.

C_F = Coons medium – KNO_3 + $(NH_4)_2CO_3$ 2 %. 25° C.

Radial Advance in mm.	Time in Days.	Type of strain originating from each successive inoculum.
	1	
	2	
	3	
	4	
7·0	5	C C C C C C C
	6	
10·0	7	F F C C C C C C
	8	
	9	
17·5	10	F F F F C C C C C C C C
	11	
	12	
25·0	13	F F F F F F C C C C C C C
	14	
30·0	15	F F F F F F F F F F C C C C C

C denotes DH_C strain.
F „ DH_F strain.

N.B. C and F recorded in a line indicate the nature of the mycelium present along any given radius of the culture.

IV. RELATION OF SALTATION TO TEMPERATURE.

Temperature has no influence upon the general character of the strain DH_C. The relation between temperature and saltation is that at a higher temperature (25° C.) the saltation sometimes appears on the 5th day, while at a lower temperature, such as 12° C., it does not take place until about the 12th day, and then not so uniformly. At a still lower temperature (3° C.) there is no change in the older mycelium. In order to confirm the last point, five sets of standard medium cultures of DH_C were placed at 3° C. after they had grown at room temperature for 4, 5, 6, 8, and 10 days respectively. When examined after definite intervals of time, the cultures did not show any appreciable growth. As to saltation it was found that no saltation had taken place in the first two sets, while with the remaining three sets the saltant zone (DH_F-zone) had not made any further progress.

Obviously temperature is only a subsidiary factor, which influences indirectly the initiation of saltation by directly affecting the metabolic processes of the fungus. Thus at 25° C., approximately, the optimum temperature for the strain, when the metabolic activity is greatest, the changed condition appears very early ; at a lower temperature the activity

becomes less and so the change is delayed; finally, at very low temperatures, when the fungal activity is almost arrested, the change does not occur at all.

V. Further Details Relating to Saltation and Saltants.

A. *Cyclic Nature of Saltation.*

Up to the present the experiments have been confined to the study of saltation in DH_C in its various aspects. A short account will now be given of saltation in the saltant DH_F.

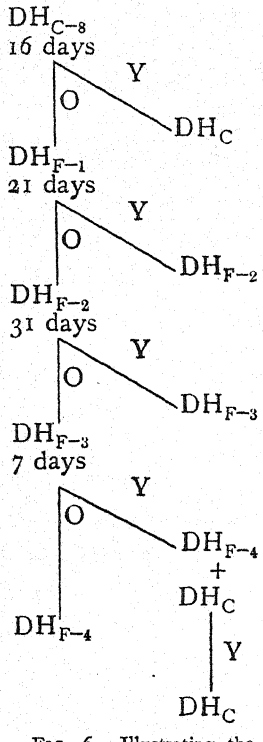


FIG. 6. Illustrating the pedigree of the culture DH_{F-3}. O, old mycelium; Y, young mycelium.

In order to find out if there is a change in the DH_F mycelium with age, the youngest and the oldest parts of a DH_F culture two weeks old, were tested in the manner previously described, but no difference in their growth characteristics could be detected. Obviously age has no effect upon the mycelial characters of DH_F.

It was during such cultural work, however, that a case of sectorial saltation was observed in the strain. DH_F had often been grown in malt agar medium on previous occasions, but nothing noteworthy had occurred, but when inocula, taken from the growing edge of DH_{F-3}—the pedigree of which will be evident from Fig. 6—were transferred to this medium, one of the cultures showed a sector having smooth mycelium, distinctly similar to DH_C, which stood out sharply by contrast with the rest of the mycelium of DH_F. In order to isolate the saltant (represented by the sector), fragments of mycelium were taken along different radial lines from the centre of the culture outwards, and also from other different positions to be grown separately in standard medium. Only one of these bred true to the parent (sector), and subsequently proved to be identical, both in morphological character and in the peculiar mode of saltation, with the strain DH_C previously obtained.

Since the inoculum from which the saltant originated was not of monohyphal origin, it may be objected that it consisted of a mixture of DH_F and DH_C, and that the sectoring which ensued was of little significance. The following considerations indicate, in the author's opinion, that the case is one of real saltation.

(1) The cultural history of the strain DH_F which had saltated (Fig. 6)

will show that, since its origin from the old mycelium of DH_C (16 days) DH_F had been subcultured for two generations, using the older mycelium of fairly old cultures (21 and 31 days), so as to make the probability of mixture very small.

(2) It is improbable that the growing edge of DH_{F-3}, which had provided the inoculum from which the saltant had directly arisen, should, in standard medium cultures, be mixed with the slower DH_C mycelium.

(3) It should be remembered that the DH_C strain was originally obtained as a saltant from a monohyphal strain of DH_B, a strain in every respect similar to DH_F.

The origin of the ever-saltating DH_C from the monohyphal strain DH_B, its continued saltation into the strain DH_F (= DH_B), and the saltation of the latter back to the parent DH_C, prove conclusively the cyclic nature of saltation in the strain. DH_C and DH_F are, therefore, parent and saltant according to whether one produces or is produced from the other. These two saltations, which are complementary to one another in the cycle of saltation, belong to very different types: in the case of DH_C the product of saltation cannot be recognized as a separate entity in the parent culture, whereas in DH_F it is a sector in sharp contrast with the parent. Further, under certain given conditions, the saltation of DH_C occurs regularly in every generation, at the appropriate age, whereas the saltation in DH_F is not an age-phenomenon at all, and cannot be foretold.

B. *Rate of Radial Growth.*

The rate of radial growth of DH_F is much faster than that of DH_C in standard medium culture, as graphically represented in Fig. 7.

At 25° C., DH_F shows a rate 3·9 mm. per day, which is twice as fast as DH_C (1·8 mm. per day); and at room temperature three times as fast (3·2 mm. against 1·0 mm. per day).

In connexion with the growth-rate, it is interesting to note that the mycelium taken from the youngest part of a fairly old culture shows a rate greater than that of mycelium taken from the oldest part.

TABLE V.

Age of the Culture.	Nature of Mycelium.	Diameter of the culture DH_F in mm. after a definite number of days.										Average Rate of Radial Growth in mm. per Day.
		3	4	5	6	7	8	9	10	11	12	
3 days	Old	20			40		52	60	70	75	90	3·75
	Young	20			40		52	60	70	75	90	3·75
5 days	Old		24		40	50	60	65		85		3·9
	Young		27		45	55	65	70		90		4·1
17 days	Old	10	20	28		42		57				3·17
	Young	20	30	40		55		69				3·8

It will be seen from Table V that the growths obtained from inocula taken from older and younger mycelia of 3 days old cultures have the same diameter throughout (90 mm.) after 12 days. But the growths from similar

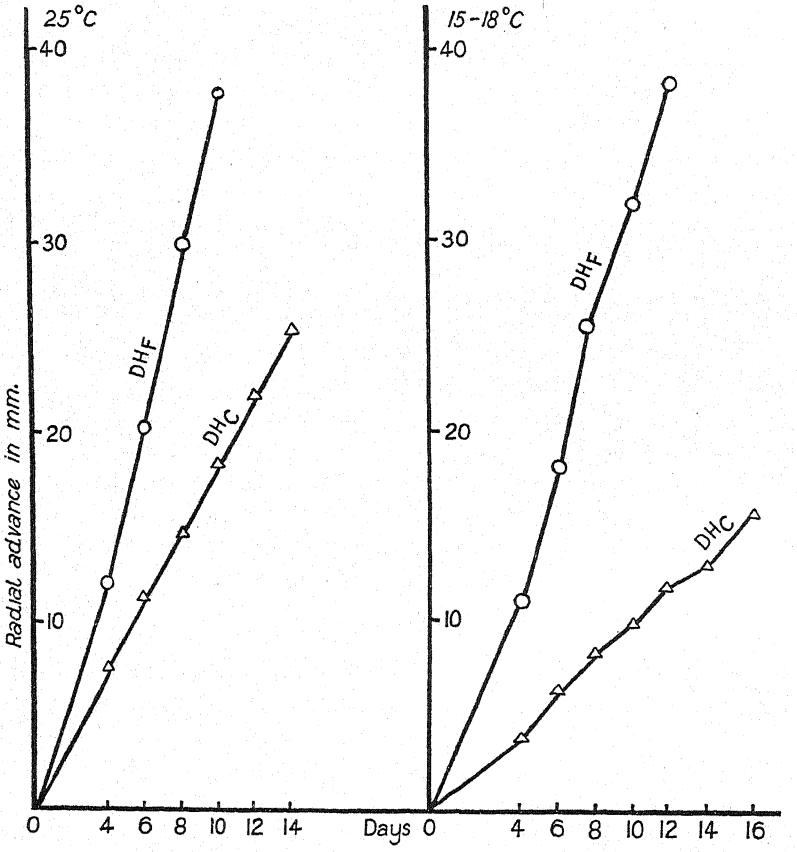


FIG. 7. A graphical representation of the difference in the rate of radial advance as shown by DH_F and DH_C at 25° C. and at room temperature.

mycelium of a 5 days old culture show a difference of 5 mm. between them (85 mm. and 90 mm. respectively). And when the growths are taken from cultures 17 days old, the disparity between their diameters is still greater (57 mm. and 69 mm. respectively, after 9 days).

It should be further noted that after 4 or 5 days *the rate of radial growth per day* is practically the same for growths arising both from old and from young mycelia, even when very old plate cultures are used. This result is easily explained. The staling products taken along with the old mycelium hamper the progress of the hyphae at the beginning; once that is overcome the rate of growth does not vary, but the ground lost at the start is hardly ever recovered.

VI. Discussion.

De Vries (4) has used the term 'ever-sporting varieties' for such forms 'as are regularly propagated by seeds, and are pure and not of hybrid origin, but which sport in nearly every generation'. Since the strain (DH_C) of *Diaporthe perniciosa* satisfies all the above conditions, except that it is propagated by mycelium, the term 'ever-saltating' has been ascribed to it. The purity of its origin is unquestionable, since it can be traced back to a single hypha, and its saltation in every generation is established beyond all doubt, although this saltation is not discernible until it is grown in a fresh medium.

How far this ever-saltating strain DH_C, which is destined to be transformed into an entirely different individual, could be regarded as a separate entity is a matter worthy of consideration. In the standard medium, though apparently unchanged, the mycelium bearing the DH_C character is really restricted only to a leading zone of a few centimetres, which continues as such so long as there is an unlimited supply of fresh medium to grow upon. No sooner is the limit reached than the loss of parental characters is a mere matter of time. In media such as Coons this character is lost earlier. In both cases the older the culture the more complete is the transformation. Furthermore, DH_C can never produce perithecia, since perithecia are produced very late in life, and so on those parts of mycelium which have changed into DH_F. The same is true with regard to the formation of pycnidia. Thus in the absence of media where DH_C will not saltate or will only saltate partially, it becomes evident that DH_C represents an immature transitory phase, a short-lived individuality as it were, whose existence is purely mycelial, and whose mature and permanent phase is DH_F; its persistence as a separate entity in nature therefore seems very unlikely.

Although not of regular occurrence, the cycle of saltation, of which DH_C and DH_F are two component phases (p. 430), is a very distinctive feature in this strain, and quite different from the unilateral or multilateral saltation shown by various fungi.

The age-phenomenon—that is to say, the incidence of saltation in the oldest mycelium at a definite stage of development, and the subsequent progressive transformation of the culture into the saltant—is a striking feature in the strain DH_C. As far as the writers are aware, this type of saltation has not been described before. A case in some respects analogous has been reported by Brown (2) in *Fusarium* D but there are, however, marked differences between the two. (1) The saltation in DH_C is definite and complete, i. e. complete transformation of the parent into the saltant, whereas in *Fusarium* D there is only a partial conversion. (2) The saltation in DH_C is continuous and progressive in a regular zone, while that of *Fusarium* D is discontinuous and irregular.

Among flowering plants this age-phenomenon finds a good parallel in 'rogue peas' as reported by Bateson (1). He states that 'Crosses between types and rogues of the same variety, however made, give F 1 in their early stages intermediate, but, *turning at or below the first flowers into rogues*, producing, that is to say, rogue-like parts at and above these levels. Self-fertilized, the plants breed permanently true to rogue.' He also states that 'in Gradus intermediate the type element commonly disappears more gradually. The change (from intermediate to rogue) is generally marked at the level of the first flower and progresses rapidly, as successive nodes are formed, so that the upper part of the plant is thorough rogue. Less often the transformation is delayed.' Though not in corresponding details, the phenomenon observed in the strain DH_C agrees very closely with this in essential features. In DH_C the change always takes place in the *oldest part of the culture*, and progresses gradually, so that the older region becomes completely transformed into the saltant DH_F, which breeds true. The parental type DH_C eventually disappears in old cultures.

The causes bringing about the saltation are, however, very obscure. It is probably associated with some cytological changes taking place in the fungus at a definite stage of development. These cytological changes, if they occur, should be open to direct investigation.

VII. Summary.

An account is given of a striking case of saltation in *Diaporthe perniciosa* obtained from the apple fruit. The saltating strain, termed DH_C, originated from another strain (DH_B) which was obtained by monohyphal culture from the original isolations of the fungus. DH_C differs from DH_B in the absence of tufted aerial mycelium, and in its slower rate of growth in certain nutrient media.

The strain DH_C is 'ever-saltating'—that is to say, a saltation into DH_F occurs regularly when cultures of DH_C reach a certain age, provided growth is not too greatly retarded by the experimental conditions. A culture of DH_C for the first few days shows no change on subculture, whatever the region of mycelium from which the inoculum is taken. After a certain period (six days at room temperature on the standard medium) inoculation from the centre of the culture is found to give DH_F, while inoculation from anywhere outside the central region gives only DH_C. The area giving DH_F increases from day to day, the circular boundary being always sharply defined. The distance between the interior circular DH_F area and the outer edge of the external DH_C margin remains constant until the original culture DH_C reaches the edge of the dish, then, as the saltation goes on, the whole culture is transformed into DH_F, and gives only DH_F on subculturing.

The regularity of the progress of the saltation is masked in certain acid media, where the saltant hyphae grow out and intermingle with the external hyphae of DH_C character. In the standard medium, the accumulation of alkali produced during growth seems to inhibit the growing out of the saltant hyphae, whereas in the acid medium the neutralization of the alkali by acid allows of the growth of the hyphae after they have undergone saltation.

The time at which saltation occurs, and the rate of the transformation of the mycelium, are influenced by temperature. Saltation occurs earliest, and the rate of change is most rapid, at the optimum temperature; at a lower temperature, both of these are delayed.

The saltant (DH_F) cannot, as a rule, be detected in saltating cultures of DH_C, since, in such cultures, the hyphae of the DH_C have apparently ceased to grow by the time they have taken on DH_F character. The existence of the saltant DH_F is, however, demonstrable on subculturing.

The strain DH_F appears to be the same as DH_B from which DH_C arose, so that the case appears to be one of saltation to parental type. DH_F usually continues true to type. Sectorial saltation, however, occurred on one occasion, the sector proving DH_C.

The ever-saltating DH_C appears to be a transitory form, having only a mycelial stage and being incapable of producing either perithecia or pycnidia; DH_F, however, is capable of producing both.

Attention is drawn to the parallelism between this ever-saltating type and the case of progressive transformation in 'rogue peas' reported by Bateson.

In conclusion, the authors wish to express their indebtedness to Professor V. H. Blackman for his kind help and criticism.

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Typhonodorum Lindleyanum: the Development of the Embryo and Germination of the Seed.

BY

L. A. BOODLE

AND

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With Plate XI and twenty-six Figures in the Text.

TYPHONODORUM LINDLEYANUM Schott, the remarkable Aroid, which grows in swamps in Madagascar, Mauritius, and Zanzibar, has been successfully cultivated for many years at the Royal Botanic Gardens, Kew, where it bears its large, white, Arum-like flowers and ripens its seeds every year.

The genus *Typhonodorum* is placed by Bentham and Hooker in the tribe Colocasieae of the Aroideae, and is considered by them to include two species, one in Madagascar the other in Mauritius. Engler, (9 and 8), however, places the genus in a separate tribe Typhonodoreae, and recognizes only the single species *T. Lindleyanum*.

The brief account in Engler's 'Pflanzenreich' is accompanied by an illustration of the plant growing in a marsh at Zanzibar and also by a page of figures (p. 71), showing an inflorescence and the flowers, ovary, embryo, and germinating seeds.

The purpose of the present paper is to give an account of the development of the embryo with details of the embryo sac and of the germination of the seed.[1]

The embryo of *Typhonodorum* is remarkable in being viviparous, like the Mangrove, and there is no resting stage in the development of the seed from the time of the fertilization of the egg-cell until the seedlings, with well-developed leaves, are dropped into the water from the pendent and swollen spathes.

The only other Aroids which produce seeds at all comparable in their

[1] The details of the earlier stages of development of the embryo have been studied and described by L. A. Boodle.

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development and structure to *Typhonodorum*, as far as can be discovered, are the species of the genus *Cryptocoryne*, a genus of twenty species of swamp-loving Aroids, found in the East Indies and Malaya, and possibly *Aglaonema palustris*, which grows in tidal waters in Malaya and Sumatra. *Montrichardia*, which grows under similar conditions in tropical America, affords no parallel to *Typhonodorum* nor does *Peltandra*, which on general grounds might be expected to have seeds showing a similar type of structure and method of germination to those of *Typhonodorum*.

Other well-known plants which have viviparous embryos are *Conocarpus erectus*, *Laguncularia racemosa*, and *Bucida Buceros* (Combretaceae), *Avicennia officinalis* (Verbenaceae), *Crinum asiaticum* (Amaryllidaceae), and the Mangroves. These are referred to in detail by Goebel (13), and they need not be further considered here.

Typhonodorum bears large, white, erect spathes, about 2 ft. 3 in. long, enclosing an elongated cylindrical spadix of about 1 ft. 9 in. in length (see Pl. XI). The spathe is constricted just above the level of the flowers, and below the constriction it is at first creamy-white and later green in colour. As the seeds mature this lower part becomes thickened, being composed of several layers of parenchymatous cells, and is closely wrapped round the lower portion of the spadix which bears the fruits. After the spathe has been expanded a few days the upper pure white expanded portion bends back and shrivels and breaks away at the level of the constriction. The peduncle then elongates and bends over, and the swollen base of the spathe hangs vertically downwards as shown in the photograph. This swollen bright green 'fruit vessel', as it may now be termed, is generally some 9 in. in circumference and may be larger according to the number of developing fruits within. When opened the spathe is found to be full of mucilaginous fluid in which the developing fruits are bathed. As a rule, under cultivation in the Tropical Water Lily House at Kew, only a few of the ovules are fertilized and produce the viviparous seeds, but if hand-pollinated most of the ovules will develop.

The Ovary. At the time of pollination the ovary has a thin, spongy wall, pink in colour, many of the cells containing a pink substance, but in the lower part the tissue is more dense and firm and the pink substance is absent. The colour gradually disappears, so that at a later stage the young seed is enclosed in a thin, white, semi-transparent pericarp, somewhat horny in consistency and ribbed, bearing at its apex a large stigmatic scar. The pericarp is a loose, bag-like envelope filled with mucilage.

The ovary usually contains either one or two ovules, but occasionally three are present. Thus, among fifty ovaries examined in this connexion, there were twenty-one with one ovule each, twenty-seven with two, and two with three ovules.

In the diagnosis of the genus *Typhonodorum* Bentham and Hooker (2)

state that there is a solitary ovule, while Engler (8) gives the number of ovules as one to two.

When the ovules are still small, e.g. 1–3 mm. in length,[1] the cavity of the ovary is usually considerably larger than the ovule or ovules in it, and the whole of the space not occupied by the ovules is filled with mucilage (Text-figs. 1 and 2, *c*). This is secreted by short clavate or cylindric hairs (Text-fig. 3), which are unicellular, or occasionally bicellular, and occur crowded together on ingrowths from the wall of the ovary. These ingrowths have the form of longitudinal ridges projecting into the cavity of the ovary, and, when prominent, suggesting incomplete septa. Text-fig. 4 is a diagram representing a transverse section of an ovary, and shows four such ridges cut across, the mucilage-hairs being omitted. The number of ridges varies in different ovaries, and often in different parts of the same ovary. Thus there may be either four or five ridges running from the apex to the base of the ovary, or one or more of the ridges present may continue for a short distance only, either starting at the apex and dying out below, or arising below the apex of the ovary by subdivision of a ridge. Owing to subdivision, as many as seven ridges may be present in parts of some ovaries.

The ridges vary in size and form, as seen in transverse section, but a common form, in the case of a well-developed ridge, has a short stalk and a broad asymmetric head, as in Text-fig. 4, *r*. One such section of a ridge with its mucilage-producing hairs is shown in Text-fig. 5. The hairs are not of uniform length, hence some of them are seen overtopping others in a section of moderate thickness. Hairs, like those on the ridges, occur on the placenta at the base of the ovary, and also in the short stylar canal.

The ridges inside the ovary are probably reduced or modified structures, representing either parietal placentas or imperfect septa, a basis for either view being supplied by members of the Colocasieae, the tribe to which *Typhonodorum* was referred by Bentham and Hooker (2). Thus a unilocular ovary with 4–6 parietal placentas is found in *Ariopsis*, while *Caladium* has a bi- to tri-locular ovary, and some of the genera have an ovary which is unilocular at the base, but bi-, tri-, or quadrilocular at the apex.

The Ovule. The ovules are basal (Text-figs. 1 and 2), erect and orthotropous, and have two integuments. Bentham and Hooker (2) give the ovule as anatropous with micropyle inferior, and Engler (8), curiously enough, also describes the ovule as anatropous with inferior micropyle, though his illustrations clearly show the ovule to be orthotropous with superior micropyle. The micropylar canal in the outer integument is, for some time, comparatively wide (Text-figs. 6–8). Its continuation in the inner integument, however, is very narrow, and sometimes practically obliterated. It may therefore be easily overlooked in longitudinal sections,

[1] In the material examined, the smallest ovule was about 1 mm. in length.

especially when these are slightly oblique. It is shown at *m* in Text-fig. 10, but is not indicated in Text-figs. 6, 8, 9, &c. The material did not include sufficiently early stages of the ovule for observing the development of the integuments.

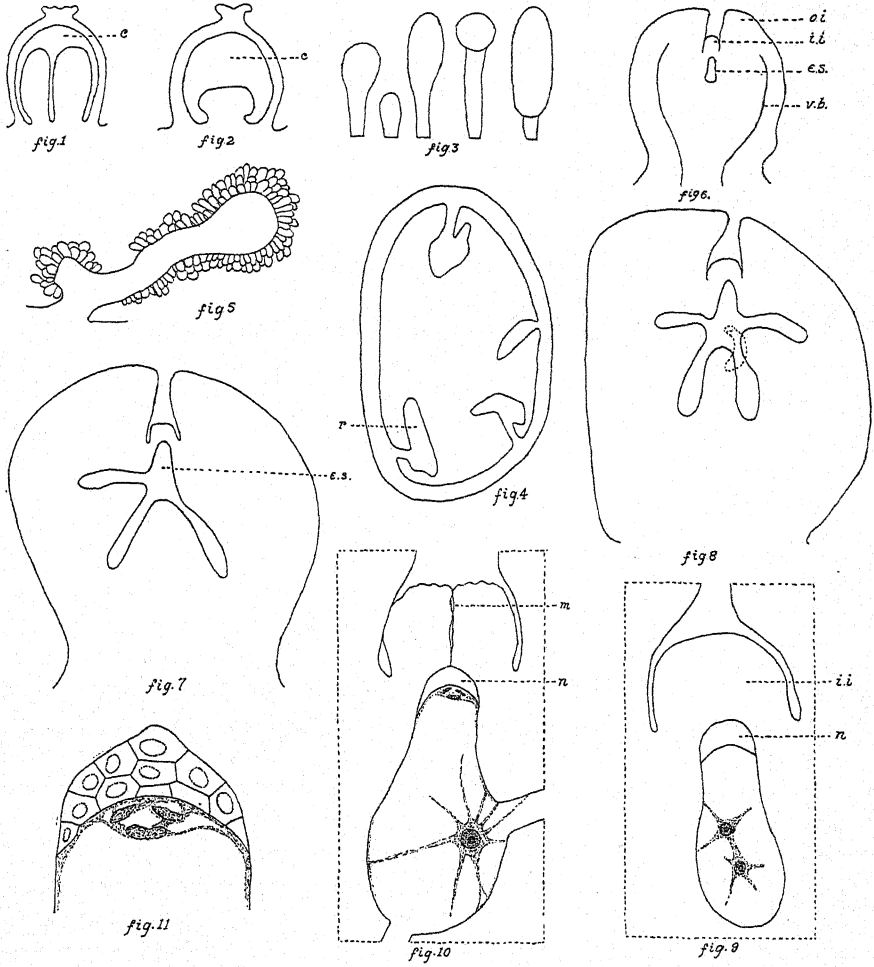
The peripheral region of the ovule is well supplied with vascular bundles, which spring from the vascular strands of the sub-placental tissue, and pass up towards the apex of the ovule. They usually die out at about the level at which the two integuments become free from one another, but some of them terminate lower. In a transverse section of an ovule nine vascular bundles were counted. Two bundles are shown in Text-fig. 6 (*v. b.*).

In the youngest ovule examined, considerable digestion of the nucellus had taken place. The nucellar tissue lateral to the embryo-sac had disappeared, but there was a cap of this tissue (*n* in Text-fig. 9), remaining at the apex of the embryo-sac, and consisting of a considerable number of cells, about twenty being counted in one longitudinal section. A rather older ovule showed a smaller cap (Text-fig. 10, *n*), with only twelve cells in the median section (Text-fig. 11). Early digestion of the lateral portions of the nucellus, leaving an apical cap of this tissue, occurs in other Araceae, as described, for instance, in *Dieffenbachia* by Campbell (4), p. 7, Pl. 1, Figs. 9 and 10.

Embryo-sac. A striking feature of the embryo-sac is the special form which it attains, owing to its producing a number of outgrowths or branches. The formation of these branches begins at an early stage, and the embryo-sac becomes swollen at the lower (chalazal) end, so that (excluding the branches), it is more or less flask-shaped (Text-fig. 10). It will be convenient to use the word neck for the narrow, and venter for the swollen portion, which becomes greatly enlarged at a later stage. The branches spread out mostly in lateral and downward-lateral directions in the ovular tissue. They vary in number, and are sometimes arranged in two tiers, each of three or four branches (Text-figs. 7 and 8). Text-fig. 7 represents a longitudinal section in which three branches are seen, while Text-fig. 8 is a diagram showing, in projection, the five branches present in one longitudinal half of an ovule, the branches lying in the other half being two in number. A stage prior to the formation of the outgrowths from the embryo-sac would appear practically as Text-fig. 9. In this case, however, two short branches had already been formed, but were not in the plane of the section represented. The space required for the growth of the branches of the embryo-sac is apparently obtained by local digestion of tissue.

The function of the branches may be in some degree haustorial. As, however, the branches neither contain nuclei, nor reach tissues specially rich in contents, indications of this function are not pronounced as in some cases of outgrowths from the embryo-sac known in other families; see Balicka-Iwanowska (1) and Coulter and Chamberlain (7).

Material suitable for studying the different stages of the contents of the embryo-sac previous to fertilization was not obtained, but the following



TEXT-FIGS. 1–11. 1. Longitudinal section of ovary containing two ovules; *c.*, cavity filled with mucilage. × 3. 2. Longitudinal section of ovary with one ovule. × 3. 3. Hairs from internal ridges of ovary. × 120. 4. Transverse section of ovary showing four internal ridges (*r.*) cut across; hairs omitted. × 8. 5. Transverse section of a ridge showing the mucilage-producing hairs. × 25. 6. Longitudinal section of ovule; *o.i.*, outer integument; *i.i.*, inner integument; *e.s.*, embryo-sac; *v.b.*, vascular bundle. × 10. 7. Longitudinal section of ovule showing embryo-sac (*e.s.*) with branches (three in plane of section). × 25. 8. Diagram of ovule showing embryo-sac with branches. The five represented (partly in projection) belong to one longitudinal half of the ovule. × 25. 9. Portion of longitudinal section of ovule showing embryo-sac with two nuclei; *n.*, nucellar cap; *i.i.*, inner integument. × 90. 10. Portion of longitudinal section of ovule; *n.*, nucellar cap; *m.*, micropylar canal in inner integument. Portions of two branches of the embryo-sac are shown. × 90. 11. Nucellar cap from the section shown in Fig. 10. Remains of egg-apparatus indicated. × 290. (All figures by L.A.B.)

observations were made. The youngest embryo-sac examined contained two nuclei, which were connected and suspended in the cavity by coarse

strands of protoplasm, the stage being shortly after the first nuclear division. The next stage seen was that of a number of ovules, which had evidently reached the receptive stage a considerable time before the material was collected, but had not been fertilized. In these the egg-apparatus showed different degrees of collapse or disintegration, but, where preservation was best, a group of three cells could be recognized, the egg-apparatus being probably of normal type. Towards the base of the embryo-sac a single nucleus, usually in good condition, was suspended by cytoplasmic threads, but no antipodal cells were found. As, however, antipodal cells sometimes do not persist for very long, they may have been originally present, and the solitary nucleus, referred to above, may be the product of fusion of two polar nuclei. Hence, there is no reason for assuming that the contents of the embryo-sac are not of the common type.

The solitary nucleus, though sometimes found in the neck, was more often placed in the venter, and, in some cases, two or three of the threads of protoplasm radiating from it were seen to extend for some little distance into branches of the embryo-sac.

The embryo-sac shown in Text-fig. 9 is one with two nuclei, while that represented in Text-fig. 10 has undergone considerable enlargement, and is an example, like those described above, in which fertilization is long over-due.

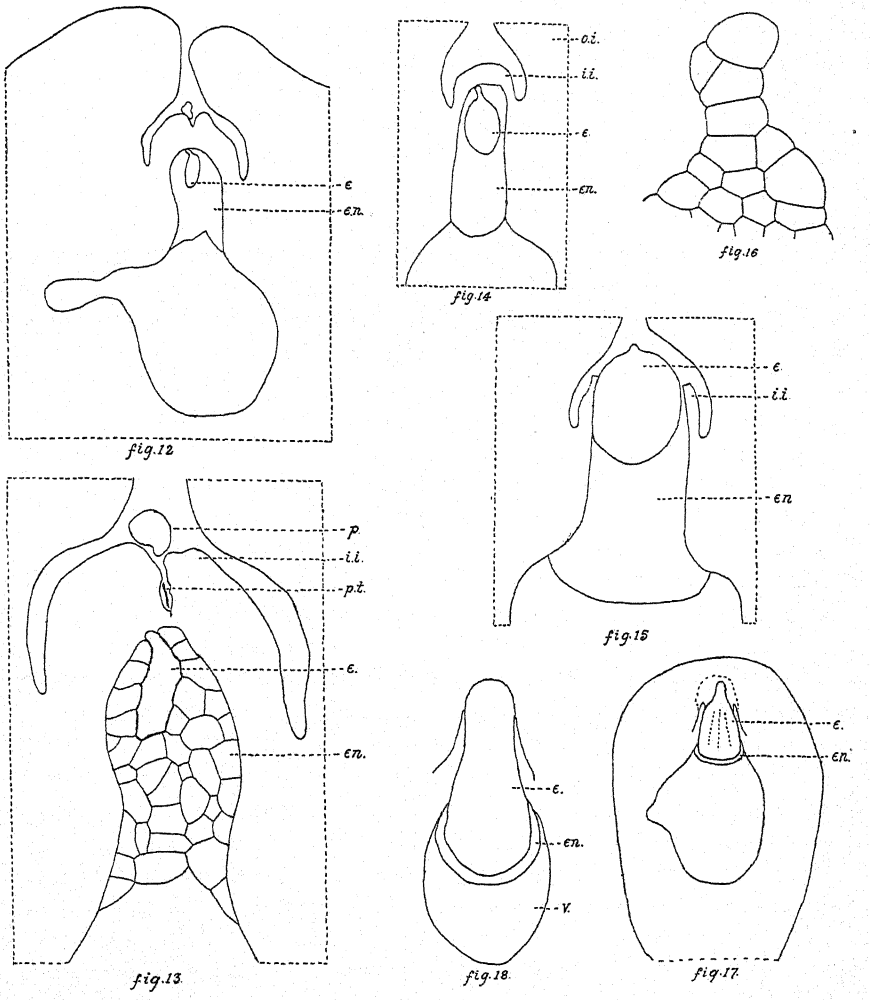
Endosperm. The endosperm is formed in the neck of the embryo-sac, and this may be the normal position of the free nucleus at the fertilization stage.

When an embryo has been formed, and is about 0·2 mm. in length, the endosperm practically fills the neck of the embryo-sac (Text-figs. 12 and 13, *en.*), forming a large-celled tissue, and showing nuclear divisions in some of its cells. There are no free nuclei adjoining the endosperm-tissue.

As no stage of endosperm-formation earlier than that shown in Text-fig. 13 was seen, evidence could not be obtained as to whether the endosperm is septate from the first, as described by Campbell (6), p. 346, in other Araceae, but it is clear that the early and complete filling of the embryo-sac with endosperm, observed by him, does not take place in *Typhonodorum.* As the embryo increases in size, enlargement of the neck of the embryo-sac takes place, with corresponding growth of the endosperm, which also grows a little further downwards, so as to project with a convex surface into the upper part of the venter (Text-figs. 14 and 15). The free surface of the endosperm occasionally consists of large, rounded, loosely connected cells, and, in one or two cases, a local downward extension of this tissue was seen on one side of the venter. By continued enlargement of the venter the branches of the embryo-sac become reduced to their distal portions.

Embryo. The smallest embryo found had reached a length of about

0·2 mm., and showed twenty-eight cells in a longitudinal section. It is shown in outline in Text-figs. 12 and 13 at *e*, and is slightly shrunken,



TEXT-FIGS. 12–18. 12. Part of longitudinal section of ovule; *e.*, embryo; *en.*, endosperm. × 25. 13. Portion of Fig. 12 further enlarged; *e.*, embryo; *i.i.*, inner integument; *en.*, endosperm; *p.*, pollen grain; *p.t.*, collapsed portion of pollen-tube. × 70. 14. Portion of ovule showing older embryo (*e.*). Lettering as before. × 25. 15. Embryo (*e.*) projecting through gap in inner integument. × 25. 16. Suspensor and adjoining cells of embryo of about the same age as in Fig. 14. × 220. 17. Section of ovule with embryo, the base of which has reached the top of the venter. × 5. 18. Embryo, which has grown well into the venter (*v.*), and is sheathed basally with endosperm. × 5. (All figures by L.A.B.).

fixation being faulty. The micropylar end of the embryo shows a definite suspensor (Text-fig. 16), a character which is not shared by the embryos of other Araceae described by Campbell. In these the rudimentary nature

or suppression of the suspensor is attributed by Campbell (4), p. 1, (5), (6), p. 329, to the fact that, at an early stage, the embryo is surrounded by endosperm tissue. The presence of a suspensor in *Typhonodorum* may perhaps be an indication that the young embryo is not similarly surrounded in this plant.

The form of the embryo at two later stages is shown in Text-figs. 14 and 15. The older embryo (Text-fig. 15), which is about two-thirds of a millimeter in length, projects some distance through a wide gap in the inner integument. This gap, though perhaps initiated by digestion of tissue, is evidently due largely to rupture and displacement of the latter, owing to enlargement of the embryo. During further development the embryo grows in length downwards, broadening at the same time, and progressively absorbing the endosperm in the neck of the embryo-sac. The base of the embryo, when it reaches the upper end of the venter, is still sheathed by a layer of endosperm (Text-fig. 17, *en.*), and this layer persists and increases somewhat in thickness while the lower part of the embryo pushes down into the venter (Text-fig. 18). Procambial strands are already present in the embryo at the stage shown in Text-fig. 17, and the rudiment of a leaf has been produced close to the micropylar end, but does not appear in the section figured.

At a very early stage the embryo is a small oblong cylindrical body with a slight protuberance on the upper side which represents the earliest stage of the plumule. This gradually develops in a horizontal direction so that the young plumule lies in a curved position at the apex of the embryo, just below the micropyle, and the leaves quickly develop, their point of insertion becoming shifted to one side of the embryo by unequal growth in the tissues of the latter. A small knob can be seen at the base of the plumule, which is the root tip, but this does not undergo any further development until the seed is nearly ripe.

In an ovule measuring 1·8 cm. long by 1 cm. broad the young embryo was 6 mm. broad by 5 mm. deep, and bore a peg-like process 2·5 mm. in length and 1·5 mm. in diameter growing down through the constriction of the sac. The lower chamber has a gelatinous lining, and besides its liquid contents, all it contains at this stage is the irregular mass of large cells, belonging to the endosperm, which are attached to the ingrowing peg-like process of the embryo. It appears that through these cells the developing embryo absorbs its supply of food from the mother plant.

The embryo develops with remarkable rapidity, and at an early stage it completely fills the upper chamber of the embryo-sac.

The main portion of the young embryo consists of a dense corm-like body on the upper side of which the plumule lies, while from the lower side of this organ the peg-like haustorial outgrowth develops. The corm-like mass of tissue, which is attached laterally to the base of the plumule, quickly

grows up on either side of the plumule forming a protective groove in which the plumule itself lies, while the margin in contact with the embryo-sac develops several blunt knobs or projections, which have grown out into the various short branches of the sac, similar knobs being eventually formed by the peg-like outgrowth. The tissue connecting the corm-like and peg-like bodies remains relatively narrow, resembling a neck.

It is after the corm has completely filled the upper chamber that the 'peg' at its lower side grows down into the lower part of the sac, where it expands into a knob-like or globular haustorial organ with a wrinkled nodular outline formed by the superficial irregular cells, through which nutriment is no doubt absorbed from the sac. When this absorbent organ is fully developed the connecting rod traversing the constricted portion of the sac and uniting it with the embryo proper appears as a very slender structure.

A section of the globular or peg-like absorbent organ shows that it is composed of large cells filled with more or less circular starch grains. The outer surface of the starch-containing tissue is well marked and ruminate, and outside this there are several definite layers of spongy absorbent tissue without starch, lying regularly over the starch-containing cells and following their crenulations like a many-layered absorbent epithelium.

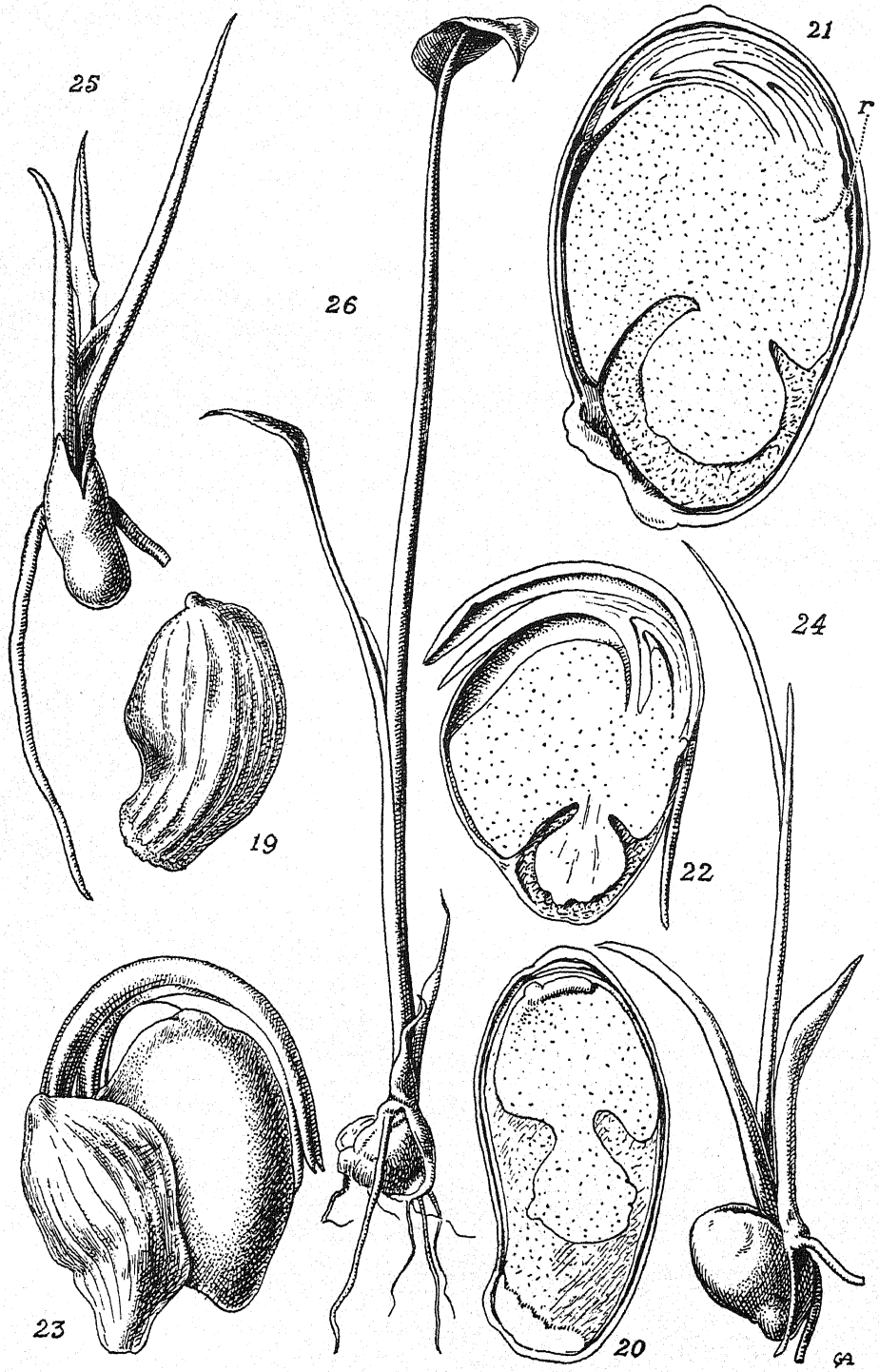
The outer cells of this tissue are papillate and full of protoplasm, in marked contrast to the storage cells of the inner portion of the 'peg'.

It seems probable that this layer of 'protoplasmic tissue' is part of the endosperm and does not belong to the embryo; as a sharply-defined epithelial layer can be detected bounding the globular peg-like projection, and as the protoplasmic layer, though closely adhering to the peg, is not in direct organic connexion with it.

Considerable interest attaches to the corm-like organ and its haustorial peg, which have so far been described without any reference being made as to their morphological nature.

The only comparable case that can be found to the embryo of *Typhonodorum* is that of *Cryptocoryne*; see Goebel (11) and (12). Here, however, there is no corm-like body such as is found in *Typhonodorum*, but only an ovoid haustorial cotyledon which gradually absorbs the endosperm in the lower part of the embryo-sac. The plumule and the rudiment of the primary root of the embryo of *Cryptocoryne* lie in a very similar relationship to the cotyledon as do these organs in *Typhonodorum* with reference to the corm-like structure.

On the analogy of *Cryptocoryne* it seems reasonable to assume that the peg-like haustorial organ of *Typhonodorum*, which penetrates and ultimately fills the lower portion of the embryo-sac, actually represents the cotyledon or a portion of it, while the corm-like body, which serves as a store of nutriment for the young plumule is a special structure, laterally developed,


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which may be regarded either as a specialized portion of the cotyledon or possibly as a lateral hypocotyledonary outgrowth.

Whatever may be its true morphological nature this compound organ performs an important function when the seed is dropped into the water.

The corm-like body acts as a float for the young seedling in addition to its being a store-house of food, but the now spongy haustorial organ has an equally important secondary function, since it enables the young seedling to float vertically in the water, with the plumule at first horizontal and later erect, so that when the seed comes to anchor it is all ready to take root and grow upwards. If the mass of spongy tissue be removed—as was the case with some seeds sent from the Seychelles—the seeds on being placed in the water will only float on their sides, and in this position they could easily get washed away and find no anchorage. It was found, however, that the seeds would float in the vertical position perfectly well when a small knob of clay, the size of the broken-off piece of tissue, was fixed to the 'corm' by a pin, thus demonstrating conclusively the important secondary function of the peg-like outgrowth.

In *Cryptocoryne* the young plant which is liberated into the water consists of two parts, the upper being the plumule with numerous leaves, a very small stem and root rudiment, while the lower part is the haustorial cotyledon, which breaks away from the young plant shortly after it has become free in the water, and the 'seedling' minus the cotyledon floats.

The young seedling *Typhonodorum*, however, retains its haustorial organ for some time after it has been shed into the water, and this enables it to come to a resting-place where it can take root in the proper position.

The mature viviparous seeds of *Typhonodorum*, when liberated from the spathe, where they have been carrying on their continuous development bathed in mucilage, are from 4·5–5·5 cm. long, 3–4 cm. broad, and 1·5–2 cm. thick, being obovate in outline and somewhat laterally compressed. The

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TEXT-FIGS. 19–23 from drawings by the late Mrs. M. Smith; TEXT-FIGS. 24–6 from photographs by G. Atkinson).

TEXT-FIGS. 19–26. 19. A young fruit, showing the white, horny pericarp, attachment scar and also stigmatic surface. Nat. size. 20. A similar fruit, in longitudinal section, showing the haustorial portion of the cotyledon, with some of the protuberances, attached to the corm-like body by a narrow neck. At the top, the developing plumule can be seen. × 2. 21. An older fruit in section, showing a considerable development of the numerous plumular leaves lying in a curved position at the apex of the corm-like body. The root apex (*r.*) can also be seen. × 2. 22. Longitudinal section of a seed which has become free from the pericarp, showing the leaves of the plumules expanding and a developing root. This was taken from a spathe, and shows the condition in which the seeds are dropped into the water. Nat. size. 23. A mature seed taken from the spathe with the pericarp still attached and hiding the haustorial portion of the cotyledon. The plumule is becoming freed from the groove of the 'corm' in which it lies when developing. Nat size. 24. A young seedling, side-view, liberated from the spathe as it floats in the water, showing the haustorial portion of the cotyledon at the base, the large obovoid, corm-like body and the young plumular leaves and roots. The young leaves show no trace of a lamina. Nat. size. 25. A similar seedling seen on edge showing the groove at the apex of the corm-like body in which the plumule lies when the seed is enclosed in the pericarp. Cf. Fig. 22 apex. Nat. size. 26. An older seedling showing the development of a lamina on the older leaves. The seedling has become rooted in the mud.

bulk of the seed is composed of the stout, dark, brownish-green, corm-like body, and is covered by the white, semi-transparent, horny pericarp (Text-figs. 19 and 20).

Lying horizontally in the groove at the apex of the 'corm', the well-developed plumule with numerous linear-subulate leaves can be seen (Text-fig. 21), and in some cases, when the seedlings are liberated, the young leaves have already burst through the pericarp and have elongated to some considerable extent (Text-figs. 22, 23).

The highly-advanced condition of the plumule in the developing embryo can be well seen in Text-figs. 21, 23, where numerous young leaves are shown, in the longitudinal section of the seedling, still enclosed in the pericarp. When the seedling is liberated the young leaves expand, and it is seen that the first three to six leaves consist of petiole only without any definite lamina (Text-figs. 24, 25). All this time, no doubt, the plumule is developing at the expense of the material stored in the corm-like body, as indicated by the rapid growth of the young leaves.

As soon as the seedlings come to rest and take root the succeeding plumular leaves grow to a considerable length, the first few of them developing laminae of lanceolate or ovate shape (Text-fig. 26), while the later ones produce the characteristic sagittate laminae which are typical of the mature plant (see Pl. XI, Figs. 1 and 2).

It should be mentioned, in conclusion, that *T. Lindleyanum*, as growing at Kew, was figured in the Botanical Magazine tab. 8307 in the year 1910. This original plant was raised from a plant sent to Kew from the Botanic Garden, Dahlem, Berlin, in 1905, which flowered for the first time in 1909. A short account, with a plate, of the Kew plant, was also published in the Gardeners' Chronicle (10) in the year 1912. In the Botanical Magazine account the plant is described as a 'shrub' with a stem, but this is hardly correct as the tall stem-like structure is a pseudo-stem, similar to that of the Banana, composed of the sheathing leaf-bases, and the actual stem is only a small conical organ 6 in.–1 ft. high.

The spathe in the Botanical Magazine plate is coloured a bright canary-yellow, but its usual colour is creamy-white, and the spadix only is yellow.

Summary.

The ovary contains one or two, rarely three, ovules. Mucilage is present in its cavity, and is formed by glandular hairs on internal ridges, which may represent modified parietal placentas.

The ovules are basal, erect and orthotropous, with two integuments, the outer one being more strongly developed than the inner.

Digestion of the nucellar tissue lateral to the embryo-sac takes place at an early stage, an apical cap of nucellus remaining for a time.

The embryo-sac produces a number of outgrowths or branches, which extend towards the periphery of the ovular tissue. The original part of the embryo-sac becomes differentiated into an upper, narrow, and a lower swollen portion.

Early complete filling of the embryo-sac with endosperm does not take place, the endosperm being restricted, for a considerable time, to the upper portion of the embryo-sac.

The young embryo has a short suspensor. As the embryo increases in size, its upper end bursts through the inner integument, while its lower part grows downwards, absorbing the endosperm around it, except for a layer of this tissue, which remains sheathing its lower extremity, when the latter pushes into the lower portion of the embryo-sac.

There is no arrest in the development of the embryo, which consists of two distinct portions, an upper dense corm-like body, at the apex of which lies the developing plumule, and a spherical spongy body which is the haustorial portion of the cotyledon. It is considered probable that the corm-like body is either a specialized portion of the cotyledon or a lateral hypocotyledonary outgrowth which acts as a store of reserves for the young seedling.

The haustorial portion has a secondary function as a 'float' enabling the seed to float upright when shed into the water.

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EXPLANATION OF PLATE XI.

Illustrating Mr. L. A. Boodle's and Dr. A. W. Hill's paper on *Typhonodorum Lindleyanum.*

Fig. 1. *Typhonodorum Lindleyanum*, growing in House No. 15 at Kew, showing the tall pseudo-stems and large sagittate leaves; young plants raised from seed are growing at the side of the parent plant. An open spathe, with erect spadix, is shown in the centre of the plant, and a number of pendent flower stalks with the swollen bases of the spathes enclosing the developing fruits. From photographs by E. J. Wallis.

Fig. 2. The upper part of the plant showing the spathe and spadix more in detail, with one flower stalk just bending over, showing the place where the upper portion of the spathe has broken away from the lower bulbous portion, and also one completely bent over and enlarged, containing the developing fruits.

Typhonodorum is now being grown in House No. 10 at Kew.



Fig. 1.

BOODLE & HILL — TYPHONODORUM.



Fig. 2.

Huth, London



Variations in Megaspore Number in Selaginella.

BY

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With four Figures in the Text.

IN the course of an anatomical investigation on *Selaginella*, a megasporangium of *S. Willdenowii* was seen to contain twenty-four small megaspores. This observation led to the examination of cones of several species, with the object of ascertaining the frequency and range of variation in megaspore numbers.

A few cases of increase in megaspore numbers in *S. Willdenowii*, *S. Lobbii*, and *S. Watsoniana* were recorded in 1927 (3). The present paper is the result of more extended observations, in the course of which cones of thirty-three species have been examined.

Occasional variation had previously been recorded in several species.

In 1901 Lyon (6) pointed out that in *S. rupestris*, never more than two spores reach maturity, and in some cases only one spore is formed, but in *S. apus* eight spores may be formed in rare cases.

Denke (2) in 1902 stated that in *S. stenophylla* four-spore mother-cells may form tetrads, but only two tetrads mature.

In 1910 Mitchell (7) described two cases of increase, one of eight spores in *S. involvens*, and one of twelve in *S. Vogelii*, in addition to reduction to three spores in *S. Bakeriana* and occasional reduction to three, two, and one spore in other species and several cases of inequality in size of spores.

Goebel (4) in 1910 found that in *S. rupestris*, only one or two well-developed spores were usually present in the sporangium, but smaller spores might be seen with them.

Kainradi (5) in 1912 described two functional tetrads, and in one case four well-developed tetrads in *S. helvetica*, as well as one case of reduction to three, and several cases of inequality in size of spores in the same species.

S. Willdenowii, Bak. supplies evidence both of increase and reduction in spore number. The cones of this species are often entirely microsporangiate, but 178 megasporangia were obtained from 118 cones.

Fourteen of these showed variations in spore number. There were four cases in which the sporangium contained only one large spore, two cases of one large and two small spores in a sporangium, one case of 16, one of 24, two of 36, and four of 42 spores.

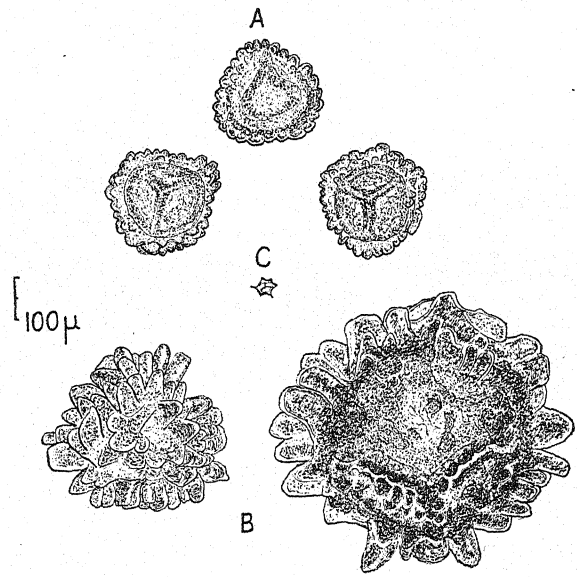


FIG. 1. *S. Willdenowii.* A, Group of three megaspores from a sporangium containing twenty-four spores; B, two megaspores from a normal sporangium; C, microspore.

The great majority of the remaining 164 sporangia contained one large and three small spores, the others either two large and two small, or more rarely three large and one small, or four equal spores.

Fig. 1, B, represents a large and a small spore from a sporangium which contained two large and two small spores.

A shows three spores from a sporangium which contained 24 spores, and C a microspore of the same species.

S. inaequalifolia, Spring v. *perelegans*, Moore. Ninety-nine cones, with the number of megasporangia varying from 3 to 32 on each cone, were examined. Two sporangia showed increase in spore numbers. In one case 36 spores were counted and in the other 24. There were also 10 cases of reduction to a single large megaspore, and one case of two equal spores.

Three of the spores from the sporangium containing 36 are shown at A in Fig. 2, while B represents one large and one small spore from a normal megasporangium. In this species the megasporangium usually contains two large and two small spores, or, in rare cases, one large and three small spores.

S. Lobbii, Moore. 140 cones of this species provid[illegible] 330 mega-

sporangia, and these furnished a number of examples of increase in megaspore numbers.

There were twenty-three cases of 8 spores, twenty-eight of 12, nine of 14, ten of 16, one of 18, two of 20, and three of 24.

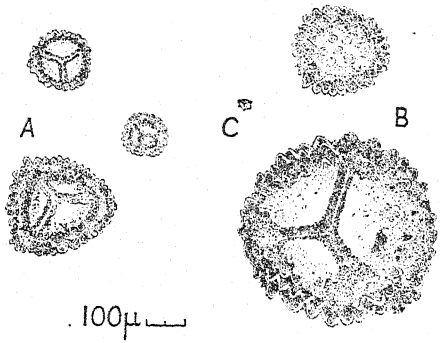


FIG 2. *S. inaequalifolia* v. *perelegans*. A, group of three spores from a sporangium containing thirty-six megaspores; B, two megaspores from a normal sporangium; C, microspore.

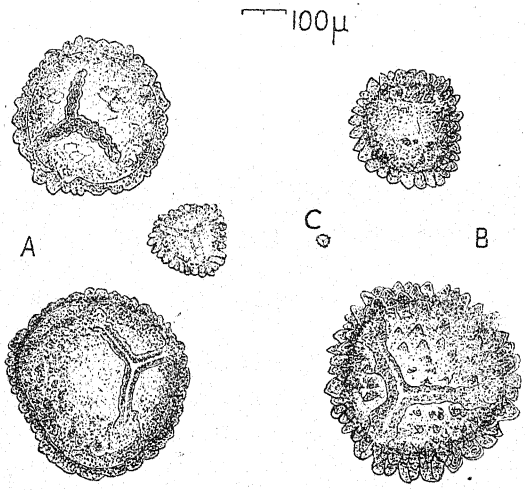


FIG. 3. *S. Lobbii*. A, group of three megaspores from a sporangium containing twelve; B, two spores from a normal megasporangium; C, microspore.

In this species, as in *S. inaequalifolia* v. *perelegans*, the megasporangium usually contains two large and two small spores, and more rarely one large and three small spores. One of each type of spore from a normal sporangium is shown at B in Fig. 3, and at A three megaspores from a sporangium which contained 12.

It is noticeable that the diameter of the largest abnormal megaspore is almost as great as that of the larger of the normal spores.

In cases where the number of megaspores is not a multiple of four, it is

evident either that some spore mother-cell has divided only once, as Lyon (6) showed to be the case in *S. rupestris*, or as Mitchell (7) suggests for cases of reduction, that tetrad divisions have occurred, and some of the resulting cells have failed to develop into spores.

S. Watsoniana, hort. Sandes.[1] Sixty-three cones, with 157 megasporangia, were examined. Two sporangia contained 8 spores. Seven

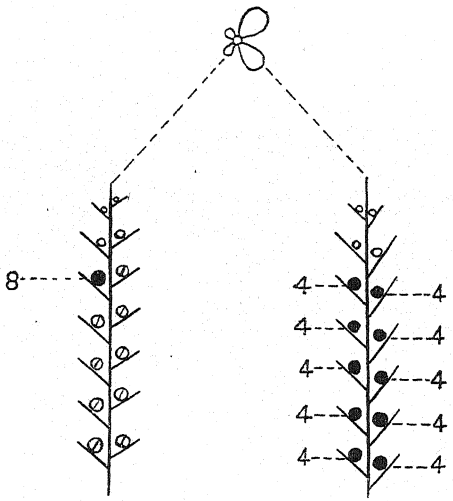


FIG. 4. Diagram showing distribution of the sporangia on the cone in *S. serpens*. o = Young sporangium; ⌀ = Microsporangium; ● = Megasporangium. The numbers indicate the number of megaspores in each sporangium. The left- and right-hand figures represent halves of a single cone in line with the small dorsal and large ventral leaves respectively.

sporangia contained two large and two small spores, and two contained one large and three small spores. In the majority of cases, however, the megasporangium contained four spores of equal size.

S. serpens (Desv.), Spring. Twelve cones of this species were examined, with a total of 131 megasporangia. In three cases a megasporangium was found to contain eight spores. In two sporangia there were two large and two small spores, and in all others four spores of equal size were present.

The distribution of the sporangia on the cone is interesting in this species. The vegetative leaves are of two kinds, but the sporophylls are uniform and *S. serpens* has accordingly been placed by Baker (1) in his subgenus *Stachygynandrum*. The megasporangia are distributed on the cone in line with the small dorsal leaves. Each of the three megasporangia observed to contain eight spores was in the position normally occupied by a microsporangium. In rare cases megasporangia with four spores were also found in line with the small dorsal leaves.

S. erythropus (Mart.), Spring. In this species seven cases of numerical reduction in megaspores were provided by 48 cones, with megasporangia

[1] This is a variegated form of *S. plumosa*, Bak.

varying in number from two to eleven on each. One sporangium contained one large and two small spores, four contained two equal spores, and two a single large spore. In the remaining megasporangia there were usually two large and two small spores, or, more rarely, one large, three small, or three large, one small, or four spores of equal size.

S. Bakeriana, Bailey. Forty-one cones were examined, and in three cases they were wholly micro-sporangiate. In the other cases there was a single, large, basal megasporangium which contained three spores in every case.

S. stenophylla, Al. Br. Twenty-two cones were examined, with megasporangia varying in number from nine to nineteen for each cone. One example of reduction to two spores of equal size was observed. Fourteen sporangia containing two large and two small spores were seen, but otherwise each megasporangium contained four spores of equal size.

S. chrysorrhizos, Spring. Twenty-six cones of this species were examined, the number of megasporangia on each cone varying between two and four. One sporangium was observed to contain one large and two small spores, three sporangia contained two large and two small spores each, the rest held four spores of equal size.

In *S. canaliculata*, Bak., *S. brachystachya*, Hook et Grev., *S. Hanseni*, Hieron., *S. inaequalifolia* (Hook et Grev.), Spring, and *S. lepidophylla* (Hook et Grev.), Spring, the megasporangia usually contain two large and two small spores. Only in rare cases in *S. brachystachya* were four spores of equal size observed.

S. molliceps, Spring, showed six cases of two large and two small spores in a sporangium; one case of one large and three small spores. *S. Martensii*, Spring, gave one case of two large and two small, and one case of one large and three small spores. In *S. cathedrifolia*, Spring, two large and two small spores were observed twice, and *S. integerrima*, Spring, supplied one case of two large and two small spores. In each of these four species the megasporangium usually contains four spores of equal size.

The other species examined were: *S. Vogelii*, Spring, *S. grandis*, Moore, *S. Kraussiana* (Kunze), Al. Braun., *S. Victoriae*, Moore, *S. pubescens*, Al. Br., *S. selaginoides* (L.), Link., *S. pallidissima*, Spring, *S. chrysocaulos* (Hook et Grev.), Spring, *S. caulescens* (Wall.), Spring, *S. pilifera*, Al. Br., *S. viticulosa*, Klotzsch, *S. atroviridis* (Wall.), Spring, *S. laevigata Bak. v. Lyallii Spr.* and *S. Haematodes* (Kunze), Spring. With the exception of *S. grandis*, where only microsporangia were observed, the other species showed four spores of equal size.

Discussion.

The present investigation has brought to light an extent of variation in megaspore numbers in *Selaginella* beyond that hitherto recorded.

Although the variation is sporadic it occurs in many species, and examination of additional species would doubtless yield further examples.

The abnormal megaspores, those from megasporangia containing more than four spores, have not yet been germinated. The possibility must not therefore be excluded that their behaviour on germination may differ from that of normal megaspores. Indeed, Goebel (4), after examining *S. rupestris* and finding the megasporangia to contain usually one or two well-developed spores, though other smaller spores may be present, suggested that the small spores might germinate as microspores. In support of this, however, no evidence was brought forward.

The occurrence in *Selaginella* of megasporangia containing many comparatively small spores, suggests a condition possibly not far advanced beyond the homosporous state, and, on the other hand, the sporangia with fewer, comparatively large spores, indicate an advance in the direction of the seed habit.

It is clear that, in the evolution of the megasporangium of *Selaginella*, mother-cells which had the capacity to develop spores have ceased to do so, until in the normal case only one spore mother-cell divides to form spores. That the other spore mother-cells have, in some cases, still the capacity to divide and form spores, is exemplified by the cases of numbers of megaspores greater than four, where more than one spore tetrad has been formed in the megasporangium.

This condition is reminiscent of *Selaginellites Suissei*, in which the megaspores varied in number from 16 to 24 in each sporangium, and *S. elongatus*, with 20 to 30 megaspores in each. Zeiller (10) was tempted to derive *Selaginella* from forms such as *S. Suissei*, by a gradual modification consisting chiefly in the progressive sterilization of sporogenous tissue of the megasporangium, so that now megaspores are formed from only one mother-cell.

The occurrence of many spores in the megasporangium of *Selaginella* is undoubtedly an atavism, pointing to the homologous nature of the micro and megasporangia.

The factor or factors inducing the divisions of more than one spore mother-cell in a sporangium have yet to be investigated. It is suggestive that all the cases of abnormal increase in spore number occurred in cultivated material, and may be the result of cultivation.

In this connexion it is interesting to observe that Shattuck (8), in cultural experiments with *Marsilia*, records the appearance of all the phases

in the development of heterospory reported by Williamson and Scott (9) for *Calamostachys Binneyana* and *C. Casheana*, and, in addition, the appearance of a megaspore of intermediate size.

In conclusion, I desire to express my thanks to Professor Dame Helen Gwynne Vaughan for much kindly interest and encouragement during the course of this work, which has been carried out in the Department of Botany, Birkbeck College.

For the material I am gratefully indebted to the Director of the Royal Botanic Gardens, Kew, the Director of the Royal Botanic Gardens, Sidpur, Calcutta, Mr. Hales, Curator of the Physic Garden, Chelsea, Mr. Stockdale, Peradeniya, Ceylon, Professor Kashyap of the Government College, Lahore, and Dr. Murray Scott of the University of California.

Summary.

The strobili of thirty-three species of *Selaginella* have been examined, and many variations from the normal number of four megaspores have been observed.

Examples of increase in megaspore numbers above four were seen in *S. Willdenowii*, *S. inaequalifolia* v. *perelegans*, *S. Lobbii*, *S. Watsoniana*, and *S. serpens*.

Reductions in megaspore numbers below four were given by *S. Willdenowii*, *S. inaequalifolia* v. *perelegans*, *S. erythropus*, *S. Bakeriana*, *S. stenophylla*, and *S. chrysorrhizos*.

Cases of inequality in size of spores were provided by seventeen species. The remaining species, with the exception of *S. Bakeriana*, showed four megaspores of equal size.

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On Carpel Polymorphism. III.

BY

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With eighty-nine Figures in the Text.

THE present account deals with the following Monocotyledon families: Typhaceae, Sparganiaceae, Potamogetonaceae, Juncaginaceae, Alismataceae, Aponogetonaceae, Butomaceae, Hydrocharitaceae. These eight families of water plants may conveniently be treated under three heads, according to certain characteristic features of the gynoecium. Upon the hitherto accepted (monomorphic) view of the carpel, the first two named above are held to exhibit the morphological contradiction of the solitary terminal carpel; the last two, the anomaly of so-called 'superficial' placentation. But upon the view that we are concerned in both these groups with *more than one type* of carpel, it at once becomes clear that the supposed nonconformity has no real existence in either case.

1. Typhaceae and Sparganiaceae (Figs. 1–32).

Gynoecium syncarpous. Ovary typically one, bicarpellary.

Typhaceae (Figs. 1–3).

Typha, the single genus of the family, has been described as having in all ♀ flowers an ovary of one carpel,[1] which in those that are functional is prolonged above into a long filamentous style which eventually widens into a slightly spathulate stigma (Fig. 1). But it is well known that many of the ♀ flowers are sterile, and in these barren forms the gynoecium differs greatly in appearance from that of the fertile organ. In both types the ovary is stipitate, but in the barren flowers the stipe becomes enlarged in its upper portion to form a massive pear-shaped structure which is surmounted by a short blunt spike in place of the long style filament (Fig. 2). Whereas, in fertile ovaries, the enlarged ovule-bearing region is situated about halfway between the stipe base and the stigma (Fig. 1). In the latter type.

[1] See Dietz, Die Blüthenorgane der Gattung *Typha* Tourn. und deren Abkömmlinge. Also a corresponding paper on *Sparganium* Tourn., Bibliotheca botanica, Schluss, p. 56, 1887.

[Annals of Botany, Vol. XLIII. No. CLXXI. July, 1929.]

a vascular bundle runs up one side of the ovary wall, and is continued for some distance up the style. A second bundle passes up the opposite side until it reaches the level of the top of the loculus. Here it turns sharply inwards to give rise to the strand of the funicle of the solitary ovule (Figs. 1, 3 A). From these facts it becomes apparent that the functional ovary is composed of *two* carpels, of which one is sterile and prolonged upwards to form the style filament and stigma, the other, fertile and destitute of both style and stigma. Starting from this interpretation of the normal ovary, we are able to discern the true nature of the above-mentioned barren, pear-shaped bodies. These are ovaries which are also bicarpellary, but in their case *both* carpels are of the sterile pattern, and the common loculus contains no ovule (Fig. 3 B). As in the fertile pistil, two vascular bundles are present and can be traced up the two opposite sides of the ovary wall, the one falling short of, the other extending up to, the top [1] (Fig. 2). At the apex the ovary suddenly contracts into a short spike derived from both carpels (Fig. 3 C), and enclosing a canal-like prolongation of the loculus. The relation of the two types of pistil thus becomes intelligible, whereas hitherto, although the forms were known, the nature of this dimorphism remained obscure.

Sparganiaceae (Figs. 4–32).

Sparganium, also a solitary genus, has hitherto, like *Typha*, been supposed to have a monocarpellary ovary, though two carpels are described as occurring in flowers here and there, two ovaries being then formed which may be more or less fused in the fruiting stage (compare Fig. 4 D).[2] But here, too, we obtain clear proof that more than one carpel is present in each of the various types of pistil often to be met with even on one individual plant. The ovary is usually conical, often becoming angular at the base through compression, with linear spathulate stigma (Figs. 4 A and B). Sections taken through a ♀ flower above the level of exsertion of the perianth members, show a main vascular bundle with several smaller veins on either side surrounding a considerable area of parenchyma (Fig. 5). At a slightly higher level branches are given off centripetally from these smaller veins, and sometimes also from the main bundle (Figs. 6, 7). These branches converge to a point opposite this main bundle and become consolidated into a single cord which at once turns horizontally to supply the solitary ovule, now visible in the developing loculus as a protuberance directed towards the aforesaid main bundle (Figs. 8, 9). From what we have learned already

[1] I have never observed in these pear-shaped ovaries an additional one or two strands mentioned by Dietz (*op. cit.*, p. 21) as occurring in some specimens. As Dietz does not describe the origin and position of these extra strands and no figure is given, it is not possible to say whether, if such cases are normal, they indicate the formation of a polymerous gynoecium, or a bifurcation of the axis such as occurs commonly in *Sparganium* (see later, pp. 461, 464, 465 and Figs. 13–30).

[2] See also Pflanzenfamilien, ii, 1, p. 192, Figs. 150, F and P.

these appearances make it clear that two carpels are present; the disposition of the two vascular systems being somewhat similar to that of the two carpel systems in *Prunus*.[1] In both cases a sterile valve carpel furnished with numerous secondary veins first takes shape, partially enveloping the later-formed solid, fertile carpel with its single unbranched (in *Sparganium*), cord. As in *Typha*, the sterile carpel alone is prolonged above to form the single style and stigma, the loculus being replaced at the top of the ovary by a core of conducting tissue (Fig. 10), and the smaller veins ceasing or becoming merged together in the style (Fig. 11), so that at the stigma level the midrib and a pair of 'wing' bundles alone persist (Fig. 12).

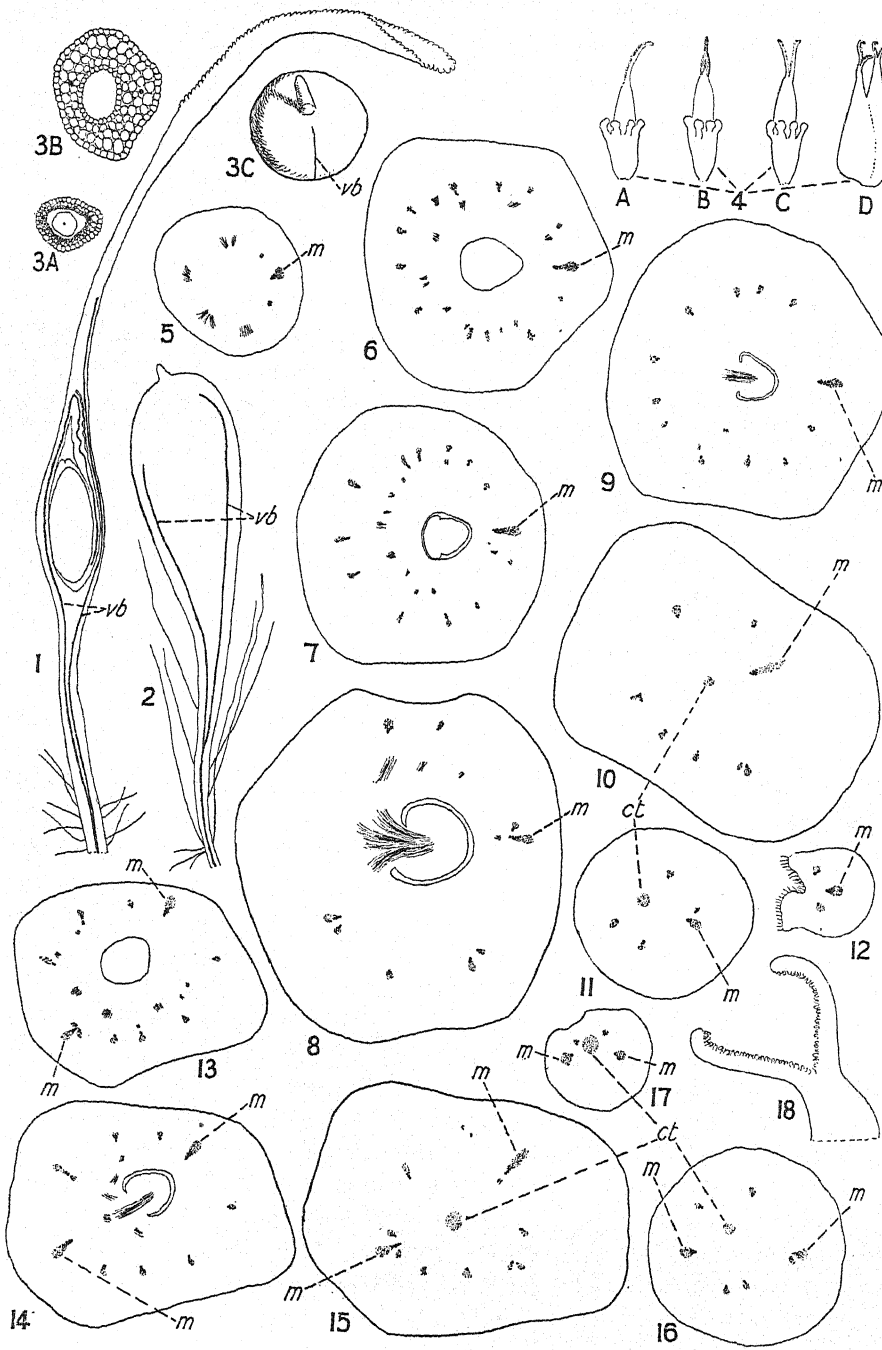
As has been noted, however, by many observers, it is not uncommon to find a normal gynoecium here and there in the closely packed inflorescences which has developed two styles and stigmas (Fig. 4 C). Eichler[2] inclined to the view that such cases resulted from the cohesion of two adjacent flowers, the pistil so formed being bilocular and containing two ovules. Dietz,[3] on the other hand, considered it improbable that these bilocular ovaries arose from the fusion of two primordia, since the two stigmas invariably face one another, and he concluded that in *Sparganium* (unlike *Typha*) the individual ovary may consist either of one carpel or of two—of one in the normal case where there is a single style, of two wherever the pistil is 2-styled. [It is essential to bear in mind that when, as here, Dietz postulates the presence of a second carpel, he has not in view the *consolidated* carpel envisaged on the polymorphic theory as always present in the normal, 1-styled gynoecium, but a *second valve* carpel in those cases only where the ovary, though really double, still appears outwardly single, as in Fig. 4 C.]

An examination of a number of these 2-styled pistils shows, however, that they do undoubtedly arise from 'twinning', but that duplication may take place at different stages in the development, and consequently gives rise to varying appearances. In extreme cases the two ovaries surrounded by a common, it may be 12-membered, perianth are barely united at the base. Or, again, they may be joined for half their height as in Fig. 4 D. In other flowers this 'twinning' does not occur until after the perianth is exserted, and its members are therefore of the normal number. The ovary may then appear to be single though actually doubled (Fig. 4 C), either *throughout*, consisting of 2 sterile valve carpels and 2 fertile carpels with 2 loculi, 2 ovules, and 2 styles and stigmas (Figs. 19–26), or *incompletely*, 2 sterile carpels being present each with style and stigma, but only 1 fertile carpel, ovule, and loculus (Figs. 13–18). In one specimen one of these 'double' ovaries showed a total of 5 carpels, 1 sterile, and 1 fertile in the one twin, and 2 sterile, and 1 fertile in the other (Figs. 27–30). It thus appears

[1] See Carpel Polymorphism, ii, Ann. Bot., xli, p. 582, Fig. 38, and p. 586, Fig. 61.

[2] Blüthendiagramme I, p. 111.

[3] *Op. cit.* p. 47.


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FIGS. 1–18. 1–3. *Typha angustifolia*, L. 1. Fertile ♀ flower rendered transparent and mounted whole. The pistil shows the vascular cord of the sterile carpel (right) extending some way up into the style; the cord of the fertile carpel (left) turns down at an acute angle to furnish the strand to the funicle. (The distal portion of the basal hairs has been cut off). 2. Barren ♀ flower similarly treated. The two vascular cords of the two carpels (both of which are sterile) are seen right and left. 3 A. Transverse section of a functional ovary in the region of the funicle. 3 B. Transverse section through the middle region of a barren ovary. 3 C. The cut-off apex of a barren ovary viewed from above, showing the short spike (pressed flat) and the terminal portion of the longer of the two vascular cords. 4–18. *Sparganium ramosum*, Curt. 4. The gynoecium, A, a single one-styled ovary showing the stigma in profile, B, the same, showing front view of the stigma, C, a single 2-styled ovary, D, twin ovaries partly fused, each with two styles. 5–12. Transverse sections from a typical 1-styled gynoecium of one sterile and one fertile carpel, taken at successively higher levels. 5. Base of the gynoecium showing the midrib (right) and secondary veins of the sterile carpel. 6, 7. The sterile carpel with midrib (right) and numerous secondary veins in a ring round the loculus; from these veins branches are being given off centripetally which later consolidate to form the cord of the fertile member. 8, 9. The same, showing stages in the consolidation of the centripetally-formed branches into the cord of the fertile member. 10. The same, taken after the loculus has closed. In the centre, a core of conducting tissue. 11. The ovary apex. 12. The style base. The numerous secondary veins have become reduced to a single pair of 'wing' bundles. The conducting tissue has opened out on to the stigmatic surface on the left. (All from the same pistil except Fig. 8.) 13–17. Transverse sections taken at successively higher levels from a single 2-styled pistil of two sterile carpels and one fertile one. 13. From below the level of attachment of the ovule. The presence of two sterile carpels is shown by the presence of two midrib bundles (one above, on the right, one below, on the left). Branches from the outer ring of bundles are seen converging towards the loculus. 14. From the level of attachment of the ovule. The single fertile cord consolidated from the centripetal bundles is seen in line with the midribs of the two enveloping sterile carpels, one of which has no fellow fertile member. 15. The same, after the fertile member has come to an end. The loculus has closed up and given place to a core of conducting cells. 16, 17. The ovary apex, showing the two midribs. In 17 only one wing bundle can be traced in each carpel. 18. The two styles from the same pistil. *m.*, midrib of the sterile carpels, *c.t.*, conducting tissue, *v.b.*, vascular bundle.

that in the typical functional ovary $G = 2$, but that through bifurcation above the perianth exsertion level, a characteristic of common though variable frequency, the number of carpels may be increased to 3 or 4, and if the process is repeated, to 5 or perhaps more. It may be remarked that this process of 'twinning' or bifurcation is less easily recognizable here than in the case of Dicotyledons. This is due to the fact that in *Sparganium* the ring of bundles which eventually persist as the midrib and secondary veins of any sterile carpel *carry out to the periphery conjoined with them the several vascular strands which later separate and become consolidated into the cord of its fellow fertile carpel.* And further, that in the basal region of such twin ovaries, there is no hint of a dividing line traversing the strip of ground tissue lying between the two consolidated fertile cords, which take up a central face-to-face position. At this level the two pistils are completely fused. Only at a higher level where they begin to separate do their contiguous boundaries become defined.

One other structural type deserves passing mention. Here and there between the closely packed functional ovaries may sometimes be found a slender, green, semi-cylindrical filament terminating in a stigma and accompanied by a bract at its base (Fig. 31). Here we have reduction of the flower carried to its utmost limit. The perianth is wholly wanting, and in place of a closed, bicarpellary ovary there is formed a single, depauperated, sterile carpel which, owing to the arrested development of the axis concerned, appears to be terminal. Though a truly terminal position for a normal foliar member is contrary to the fundamental relation of stem and leaf, it is nevertheless the case, where extreme reduction brings the axis ostensibly to vanishing point, that a reduced leaf member may be formed which has all the appearance of being actually terminal.[1]

Our analysis of the ♀ inflorescence of *Sparganium* has therefore shown the presence of the following variations in the gynoecium :

1. G = a single, sterile, styled carpel which does not form a closed ovary. Occasional, due to extreme reduction.
2. $G = 2$ carpels, one sterile, valve, with style, the other, consolidated, fertile, without style. Normal case.
3. $G = 3$ or 4 carpels (2 valve + 1 consolidated, or 2 valve + 2 consolidated), forming a single ovary with 2 styles. Frequent, due to 'twinning' above the perianth exsertion level.
4. $G = 5$ (or more) carpels forming a single ovary, generally (? always) 2-styled. Occasional, due to repeated 'twinning' as above.

[1] A somewhat analogous case is described by A. Arber in the reduced spikelet of a Grass (Ann. Bot., xlii, p. 185, Fig. 5, 1928). Where, as in this case, the foliar organ has a circular exsertion, the way in which the, to all appearance, terminal position may come about through reduction of the axis is intelligible. The case of the single *Sparganium* carpel is probably similar.

5. $G = 8$ carpels, forming 2 ovaries more or less fused, each 2-styled, due to 'twinning' both below and above the perianth exsertion level.

2. POTAMOGETONACEAE, JUNCAGINACEAE, ALISMATACEAE (FIGS. 33–54).

Gynoecium apocarpous, ovaries more than one, monocarpellary.

Potamogetonaceae (Figs. 33–7).

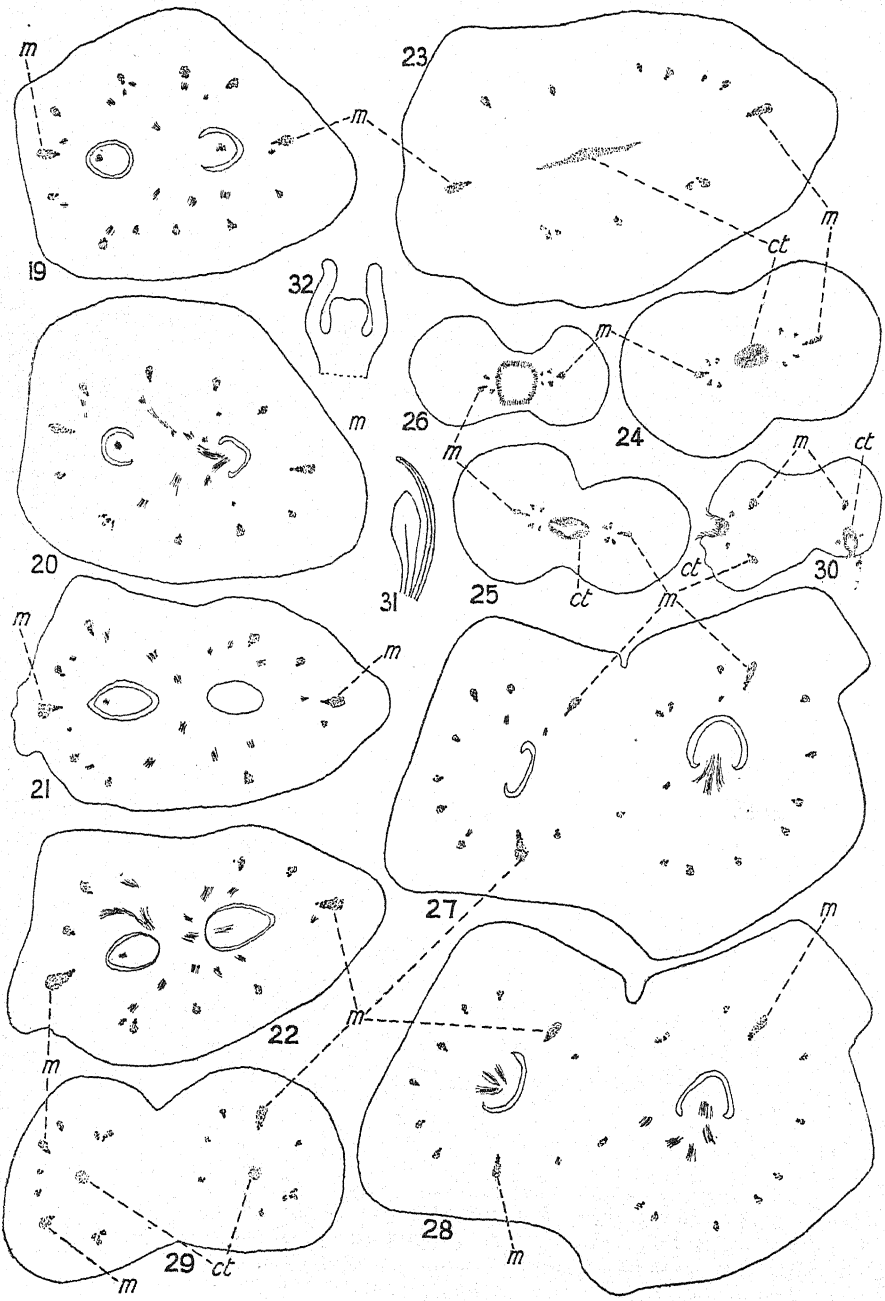
In *Potamogeton* the apocarpous gynoecium consists typically of four ovaries, each formed of a single, fertile, valve carpel. Only one carpel type being present, the formula $G\,4$ remains as before.

Although consideration of other floral whorls lies outside our immediate subject, passing reference may be made to the outer structures in the flower of *Potamogeton*. These consist of four stamens with the anther sacs of each member separated by a broad column of tissue, which develops above into a free, expanded structure showing a pinnate system of venation (Fig. 33). According to Hegelmaier[1] these expanded structures represent perianth segments fused below with the stamens. Although some more recent writers appear to hold that a perianth is wanting, a study of the vascular anatomy leaves little doubt that Hegelmaier's interpretation is correct.[2] A cross section through the base of a flower of *P. lucens* L. shows that four stout vascular cords leave the central cylinder in the orthogonal planes and, passing out horizontally, enter these outer structures (Fig. 34). Here the cords branch, the median portion, continuing in a horizontal direction, is prolonged into the expanded, free appendage representing a perianth segment (Fig. 35), and here gives off several lateral veins. The two lateral portions remain in the connective and appear as a double bundle (Fig. 35). The above-mentioned four cords are evidently trunk cords, each representing the conjoined midribs of a perianth segment and a stamen, and thus the wide separation of the anther sacs and the occurrence of a double bundle in the connective proper are explained.

The central vascular tissue remaining after the exit of the combined perianth and stamen bundles becomes consolidated into four (or fewer) cords on the diagonal radii (Fig. 34), each of these cords passing out entire to become the midrib of a carpel, and giving off a single placental strand (Figs. 37 A, B, C, D).

[1] Bot. Zeit., 1870, p. 281.

[2] It is true that this interpretation accepts the appearance of superposition of one set of structures upon another for the undeniable actuality that it is. That is to say, this view admits a transgression of the law of alternation of successive whorls. The circumstances which have led to this exceptional relation are, however, obvious. As the vascular bundle for each perianth segment carries out to the periphery conjoined with it the bundle for the stamen, these two sets of structures are necessarily superposed. On the other hand, the whole of the vascular tissue standing on the alternative radii is retained in the centre for the carpels. Accordingly no other members are formed on this set of radii.


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Figs. 19–32. *Sparganium ramosum*, Curt. (*continued*). 19, 20. Transverse sections from a single 2-styled pistil of two sterile and two fertile carpels. 19. From near the level of attachment of the ovules and before consolidation of the centripetal branches into the cords of the two fertile members. 20. At the level of consolidation of some of the centripetal branches into the fertile carpel cord on the right. 21–6. The same from another flower. 21. From a slightly lower level than 19. 22. Showing a stage intermediate between 19 and 20. 23. The loculi have closed up, and the two fertile carpels have come to an end, the centre being now occupied by a narrow strip of conducting tissue. 24. The secondary veins in the sterile carpels are much reduced in number. A slit has appeared in the central core of conducting cells. 25. The secondary veins are still further reduced in number. The slit in the conducting tissue has enlarged forming a common stylar canal. 26. The two sterile carpels are about to separate to form the two free styles; the common stylar canal is lined with stigmatic hairs. 27–30. Transverse sections from a double 4-styled pistil; the left ovary is composed of two sterile carpels and one fertile one, the right, of one sterile and one fertile carpel. 27. At the level of consolidation of the centripetal veins to form the main cord of the fertile carpel of the ovary on the right. 28. Showing the same stage in the ovary on the left. 29. Both fertile carpels have come to an end, and each loculus has given place to conducting tissue. 30. The stylar canal in the right-hand ovary has opened on to the surface between the two 'wing' bundles of the single sterile carpel; the common canal in the left-hand ovary has opened out between one wing bundle of one sterile carpel and the adjacent wing bundle of the other. 31. A depauperated flower reduced to one sterile carpel (right) in the axil of a bract (left). 32. Diagrammatic drawing of a bilocular gynoecium halved longitudinally (after Dietz). See reference in text, p. 461. *m.*, midrib of the sterile carpels, *c.t.*, conducting tissue.

Juncaginaceae (Figs. 38–44).

Triglochin (Figs. 38–43). This is perhaps the most interesting type in this whole series from the present viewpoint, for within the limits of this one genus we can trace the gradual process of reduction and degeneration of a whole carpel whorl. All the carpels are of the valve type, hence the current formula for the gynoecium ($G = 3$ or $3 + 3$) needs no alteration.

In *T. maritimum* L. (Figs. 38–41) the two carpel whorls are fully developed and fertile, each carpel having a midrib which continues to the top, and a placental strand which ceases at the level of origin of the ovule. As representing the other end of the scale, we may cite *T. Barrelieri*, Loisel. (Figs. 42, 43), in which the three antepetalous carpels are fully developed, while the three antesepalous members have disappeared almost without trace. In the specimen here figured a last remnant of one of these members is perhaps indicated in transverse section by a small convex bulge of non-vascular tissue between two of the functional carpels (Fig. 43), an appearance recalling that observable on a larger scale in species of *Tropaeolum* (*T. pentaphyllum*).[1] Between these two extremes intermediate stages occur, as e. g. in *T. palustre*, L., and *T. striatum* var. *montevidense*, Buchenau.[2]

Scheuchzeria palustris, L. (Fig. 44). The gynoecium consists typically of six valve carpels as in *Triglochin maritimum*, but the members of the inner whorl are sometimes infertile. In the specimen here figured five carpels only were present, of which four at least were fertile.

Alismataceae (Figs. 45–50).

Alisma Plantago, L. (Figs. 45–7). Carpels, usually eighteen, in a single whorl. After the differentiation of the stamen bundles the residual vascular tissue consists of a ring of eighteen cords. Each of these cords turns out to enter one of the ovaries (Figs. 45, 46), branching below the loculus to give rise to a midrib and a single placental strand. The midrib continues for some distance up the outer wall of the ovary; the placental bundle comes to an end as it furnishes the strand of the funicle (Fig. 47).

Sagittaria montevidensis, Cham. and Schlecht (Figs. 48–50). Carpels ∞. As in *Alisma*, the carpel midrib gives off a single placental strand (Figs. 48, 50). A transverse section taken through the extreme apex of the flattened axis of a ♀ flower, shows that all the remaining xylem-containing bundles, one of which turns out into each ovary, are eventually used up in this way, only perhaps a few anastomosing strands of undifferentiated vascular elements being discarded at the last (Fig. 49). The same complete

[1] See New Phytologist, 27, p. 210, Fig. 53, 1928.

[2] See Eichler, Blüthendiagramme, i, p. 101, Figs. 46, A and B. Also Saunders, Ann. Bot., xxxvii, p. 476, and Figs. 56, 57, 1923; A. Arber, Monocotyledons, p. 198, Fig. *cl. i*, G, H, 1925; Pflanzenfamilien, ii, 1, p. 224, Fig. 170 A.

utilization of the ultimate vascular units is seen in those of Ranunculaceae (*Anemone*, *Clematis*), and Rosaceae (Potentilleae, Roseae),[1] which similarly possess numerous monocarpellary ovaries.

3. Aponogetonaceae, Butomaceae, Hydrocharitaceae (Figs. 51–89).

Flower hypogynous, gynoecium apocarpous except at the base, or flower syngonous,[2] gynoecium syncarpous.

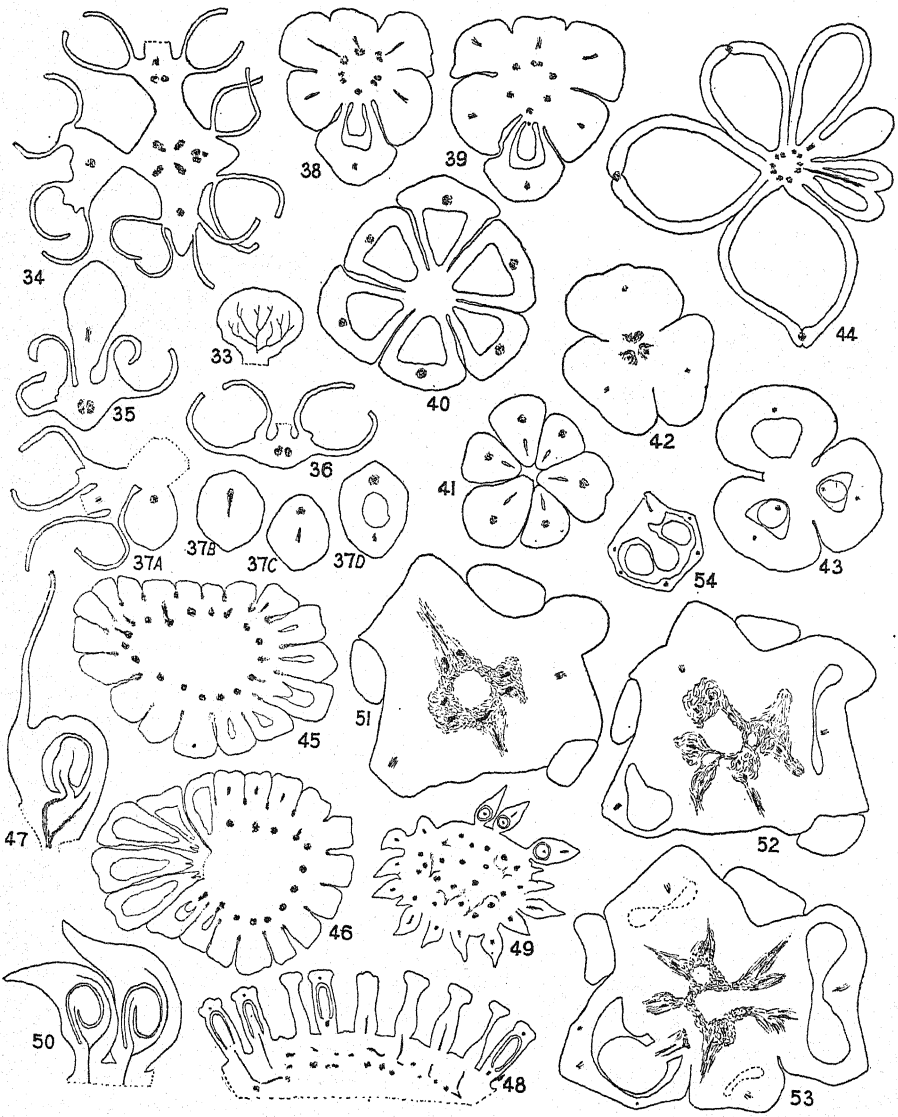
Aponogetonaceae (Figs. 51–4).

Aponogeton, the only genus in the family, has an apocarpous gynoecium usually of three ovaries, but in *A. distachyum*, Thunb. the number ranges from six to two. Though the outward appearance of the pistil is like that of *Potamogeton*, its construction is less simple and indeed presents some difficulty in the way of analysis, the complex arrangement of the large amount of residual vascular tissue differing from that of any type so far investigated, though a somewhat similar complexity is met with in the Butomaceae (see later). Taking all the evidence together, however, it seems fairly certain that the gynoecium is derived from two carpel whorls, but that the stage in which the vascular system of the inner, fertile whorl exists as so many independent midrib cords is transitory, or even non-existent, owing to the immediate breaking up of each apportioned vascular mass upon the giving off of the branches which, here, pass out into the lateral walls of the ovaries, at this level not yet disjoined.

After the emergence of the perianth and stamen cords the remaining vascular tissue is seen in a cross section of *A. distachyum* as a broad, continuous ring enclosing pith. From this ring there pass out on the same radii as the loculi as many vascular cords as there are ovaries, leaving behind again a central ring or plexus in which the elements for the most part run more or less horizontally, sometimes enclosing isolated groups of parenchyma cells as well as a central pith. These outgoing cords become the midribs of an outer whorl of sterile carpels and form no placental branch. From the central plexus still remaining twin branches now extend radially outwards between the loculi, furnishing the secondary venation in each case of the common side wall of the ovary to right and left, and also supplying the ovules borne on either side of this common wall. In this species the ovules are so crowded together at the base that they are disposed in each loculus in more than two ranks—another strong argument in addition to that derived from the vascular system in favour of the presence of an inner, fertile, consolidated carpel whorl. It thus appears that, whereas

[1] See Carpel Polymorphism, ii, Ann, Bot., xli, pp. 613, 614, and Figs. 189–193, p. 612.

[2] 'Syngonous' is used in place of 'epigynous' of the older terminology (see New Phytologist, 24, p. 185, 1925).


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Figs. 33–54. All from transverse sections taken at successively higher levels except 33, 47, and 50. 33. *Potamogeton natans*, L. Perianth segment showing venation system (after Hegelmaier). 34–7. *P. lucens*, L. 34. The flower base after the emergence of the four trunk cords, each of which passes out into one of the compound structures consisting of a perianth segment and a stamen fused together. The residual vascular tissue serving the gynoecium consists of four inner cords, one cord passing to each ovary. 35. A stamen still attached to the perianth segment, showing the double vascular bundle of the connective. 36. The same, now free. 37 A, B, C, D. An individual ovary, A, still attached to the axis with single unbranched vascular cord; B, C, showing origin and course of the placental branch; D, after the formation of the loculus. 38–41. *Triglochin maritimum*, L. 38. The gynoecium of six ovaries in different stages of development. The ovary most developed (below) shows midrib, placental strand, loculus, and ovule; in the one least developed (above) the midrib is just about to leave the central cylinder; in the others the midrib is seen running out horizontally beneath the loculus, leaving behind the placental strand. 39. The same, after all six midribs have reached the periphery. 40. The same, showing the ovaries united at the centre but otherwise free. The midribs still persist, but the placental bundles have come to an end. 41. The same, after the ovaries, still with midribs, have become completely disjoined. The loculi are closing up. 42, 43. *T. Barrelieri*, Loisel. 42. The gynoecium of three ovaries, each with midrib and placental bundle, below the level of the loculi. 43. The same, after the formation of the loculi. The placental bundle has been used up in providing the strand to the ovule. 44. *Scheuchzeria palustris*, L. Fruit of five ovaries, of which four were fertile. Each ovary shows a midrib and twin placental strands. 45–7. *Alisma Plantago*, L. 45. Gynoecium with ovaries more developed on some sectors than on others. In the younger ovaries the midrib is seen running out to the periphery, leaving the placental strand behind. In those which show a loculus the midrib is no longer to be traced. 46. A somewhat later stage from another flower. In the older ovaries both midrib and placental bundle have already come to an end. The dotted areas shown between some of the ovary bases here and in 45 indicate glands. 47. A single ovary rendered transparent viewed from the side, showing the whole course of midrib and placental branch. Lignification of the latter strand begins midway and extends in both directions. 48–50. *Sagittaria montevidensis*, Cham. and Schlecht. 48. A section of the axis of a ♀ flower showing nine ovaries. The axis shows concentric rings of bundles; a strand from a bundle in the outermost ring passes to each ovary. 49. The summit of the same axis showing the last remaining vascular bundles, each of which passes into an ovary. 50. Two ovaries showing midrib and placental strand (treated as in 47). 51–4. *Aponogeton distachyum*, Thunb. 51. The flower base showing three attached stamen bases and the vascular tissue for the gynoecium. The cords which turn out to form the midribs of two of the outer carpels can be seen leaving the centre (above on the left and below on the right); those of the other two outer carpels have already reached the periphery; in the centre the remaining vascular tissue forms a plexus enclosing a central patch of parenchyma. 52. A later stage of the same. The loculi have now appeared in line with the two earlier outgoing cords (seen on the left and right, respectively). The other two lagging cords have now reached the periphery. The residual vascular tissue is still in the form of a plexus, with islands of parenchyma here and there. 53. A later stage showing strands leaving the central plexus to extend down the side walls of the four ovaries which are still united with the axis and with each other. 54. A single ovary showing the ovules in three ranks.

Potamogeton has a single whorl of fertile, valve carpels, each with a midrib and single placental branch, the ovaries otherwise being without secondary veins, there are in *Aponogeton* two carpel whorls, the lateral walls of the ovaries being supplied by lateral veins from the inner, fertile whorl, and the ovules being sometimes developed in more than two ranks. For *A. distachyum* we may then say that *G* most frequently = 4+4, but may range from 2 to 6+2 to 6.

Butomaceae (Figs. 55–65).

The gynoecium, nearly or quite apocarpous, is described as consisting of six to eight carpels, and as having the ovules distributed over the inner face of each carpel *except along the dorsal line and margins*, a disposition not easy to reconcile with the former (monomorphic) view that only carpels of the valve type are present, but offering no difficulty if the ovaries are conceived to be, not monocarpellary, but composed each of ½ 1 ½ consolidated carpels—a construction which, as has been already shown, occurs in various Dicotyledon types, among which certain of Crassulaceae and Saxifragaceae may be cited in addition to those previously described.

Butomus umbellatus, L. (Figs. 55–8). *G* 6+6 (hitherto regarded as *G* 6). The plan of construction of the gynoecium in *Butomus* can be derived (in imagination, not as an assertion of phylogenetic connexion) from that of a liliaceous type having 'septal' glands, if we suppose both carpel whorls of the latter type to become hexamerous (instead of trimerous), and the 'septal' glands to extend to the outer surface throughout almost their whole length, so that the gynoecium becomes apocarpous. For in the liliaceous type with 'septal' glands the antepetalous carpel cords split up into twin series of strands, one series extending outwards in radial order along each side of the gland.[1] A similar branching of the fertile carpel cords takes place in *Butomus, but here the whole branch system is capable of bearing ovules*, whereas in Liliaceae only one pair of laterals is fertile. The side walls of the ovaries are glandular on their outer surface, so that were they to become coherent at their outer borders from the base up, these glandular surfaces would be comparable with the 'septal' glands of the Liliaceae. The alternate, solid carpels, having their midribs in line with the loculi, are sterile, as in the Liliaceae, the mid-line of each ovary being free from ovules throughout. A similar formation by consolidated carpels of numerous fertile strands in series is seen among Dicotyledons in such types as *Papaver*. In *Papaver*, however, the solid carpel cords *turn out from the centre*[2] to

[1] In genera lacking these 'septal glands' (*Fritillaria, Tulipa, Lilium*) the corresponding cords continue as recognizable main bundles to the top of the gynoecium, giving off only one placental strand right and left, in place of a radial series.

[2] That is to say, they did so, we must suppose, originally, but in the species of to-day the connexion of these carpel cords with the axial vascular cylinder is not differentiated at this stage. See Illustrations of Carpel Polymorphism, i, New Phyt., xxvii, p. 52, 1928.

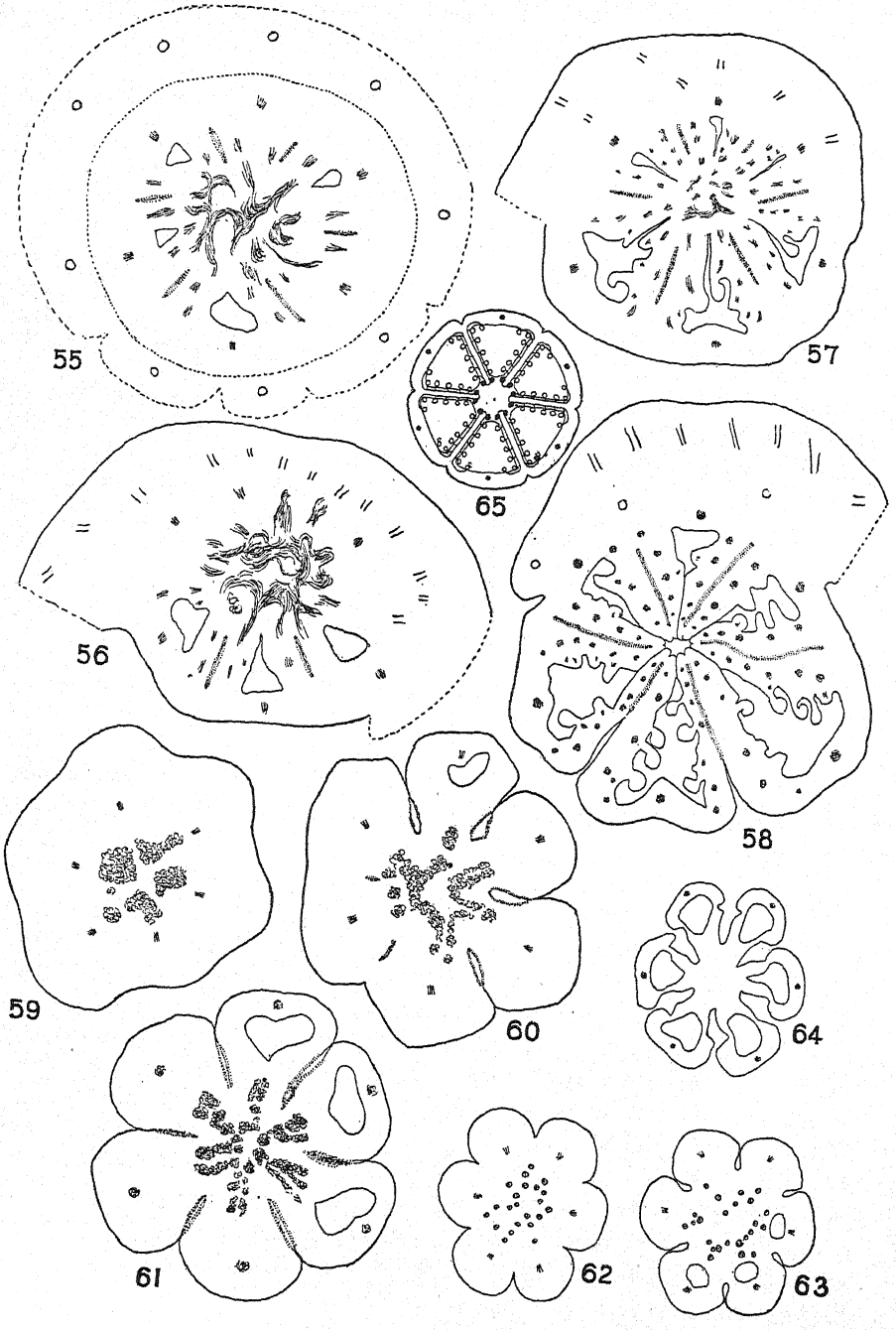
run in the outer wall of the syncarpous ovary, hence the order of origin of the radial series of fertile strands is from the outer (midrib) end, of the radial partition (solid carpel) inwards. In *Butomus* the fertile cords *remain central*, and the order of formation is from the central (midrib) end, of the partition (semi-solid carpel) outwards.

A transverse section through the base of the gynoecium shows a central vascular plexus from which the cords which become the midribs of the outer six sterile carpels pass out in line with the six perianth segments, and beneath the six loculi (Figs. 55, 56). From the plexus still remaining, at points lying on each alternate radius, i.e. at those points at which we should expect the cords of an inner carpel whorl to be situated, there extend outwards a pair of strands, one on either side of the glandular slit.[1] From these twin strands branches are given off in succession along the whole extent of the side walls of the loculi to supply the ovules (Figs. 57, 58). It is no doubt this gland conformation which causes a precocious breaking up of the vascular mass apportioned to each inner carpel, so that the stage of a single consolidated cord is extremely transitory if it can even be said to exist. The residue of the plexus ramifies in the pith and is discarded. The pith then very shortly comes to an end, the glands open on the surface, and the ovaries thus become disjoined.

Hydrocleis (Figs. 59–64) exhibits the same general construction of the gynoecium as *Butomus*, though differing in certain details. In *Hydrocleis Commersonii*, Rich. (*Limnocharis Humboldti*, Rich., *Hydrocleis nymphoides Buchen.*) which is dioecious, both ♂ and ♀ flowers possess organs of the other sex. In the ♀ flowers the septal glands open on to the surface at once, but as the pith persists for a considerable distance above the level of emergence of the sterile carpel cords, the ovaries remain for some time attached by their inner face after they become free from each other laterally.

A transverse section through the gynoecium base shows an outer whorl of six vascular cords which become the midribs of the sterile carpels. The residual vascular tissue consists of large irregular masses in which the elements run more or less horizontally (Fig. 59). These masses shortly break up. Some elements enter the pith and are discarded. From this re-arrangement no very clear pattern emerges such as one meets with in general. One can, however, make out an indication of V-shaped masses with the apex of the V abutting on the pith, and the divergent arms straddling the inner end of the septal glands in such a way that one arm comes into contact with the arm of an adjacent V on one side, and the other merges similarly with a neighbouring arm on the other side. From these arms a twin series of bundles extends radially outwards, as in *Butomus*, one on either side of the gland region. Branches from these bundles run to

[1] In this region the gynoecium has not yet become apocarpous.


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Figs. 55–65. Butomaceae. All from transverse sections taken at successively higher levels except 65. Figs. 55–8. *Butomus umbellatus*, L. 55. Gynoecium base, not yet wholly free from the conjoined stamen bases. Four of the six loculi have made their appearance. The sterile carpel cords are seen near the periphery on the radii of the loculi. The vascular system of the fertile members is still in the form of a central plexus from which branches extend outwards on either side of each septal gland (shown as a dotted streak). These branches furnish the strands to the ovules. 56. The same, partly conjoined with the perianth from another flower, unequally developed on the two sides. 57. The same, showing the numerous vascular strands supplying the ovules disposed along either side of the septal glands. 58. The ovaries are becoming disjoined through the opening up of the septal glands on to the exterior, and the gradual disappearance of the pith, which only at the last becomes free from discarded vascular tissue. The ovules are borne everywhere on the walls of the loculi except over the sterile carpel midribs. 59–64. *Hydrocleis Commersonii*, Rich. 59–61. From a ♀ flower. 59. Gynoecium base. The six sterile carpel cords have left the centre; the vascular elements for the inner fertile whorl appear in the form of irregular masses. 60. The same after the septal glands have made their appearance, and in some cases have already opened on to the exterior. The vascular masses seen in 59 after having formed a plexus are now becoming constituted into V-shaped masses straddling the central end of each septal gland, each mass constituting the vascular system of one fertile carpel. 61. The V-shaped vascular masses break at the point of the V, and the two limbs of each V diverge so as to come into contact with the adjacent limb of a neighbouring V. Three of the loculi are already in being, the other three are not yet apparent. 62–5. From a ♂ flower. 62. Base of the infertile gynoecium showing the vascular cords of the six outer sterile carpels, and numerous scattered vascular strands in the central parenchyma. 63. The same, as the loculi begin to appear. 64. The same, with loculi fully developed but destitute of ovules. The sterile carpel cords alone persist, all the central vascular strands having come to an end. 65. Diagram of the gynoecium base in Butomaceae, showing the vascular system of the six outer and six inner carpels.

the numerous ranks of ovules (Figs. 60, 61). Thus in both *Butomus* and ♀ flowers of *Hydrocleis*, the evidence points to a ground plan of two carpel whorls, the outer of solid, sterile carpels, the inner of semi-solid and fertile carpels. By the opening out of the 'septal glands' on to the surface the fertile members are split in half, and the gynoecium becomes apocarpous, the ovaries consisting each of $\frac{1}{2}$ 1 $\frac{1}{2}$ carpels.

In the gynoecium of the ♂ flowers the six outer carpels are present and form loculi, but the ovaries are barren, for the residual vascular tissue remains in the form of small scattered strands in the pith (Figs. 62, 63) which never become concentrated into the regular system of a fertile carpel whorl, but soon cease (Fig. 64). In other words, the ovary of the ♂ flower is sterile because the inner, fertile carpel whorl is not differentiated.

Hydrocharitaceae (Figs. 66, 89).

Flower syngonous,[1] gynoecium either unilocular from the first, or only towards the apex. The placentae are described as parietal, generally reaching to the middle of the ovary but not uniting, and bearing the ovules scattered over the surface. Stigmas often more or less deeply bifurcate.

Of the five genera examined, viz. *Blyxa*, *Hydrocharis*, *Stratiotes*, *Trianea* and *Vallisneria*, *Blyxa* shows the simplest arrangement.

In the ♀ flower of a form passing under the name of *B. flava* (Figs. 66–69), the ovary, which is trilocular at the base, later becomes unilocular. A transverse section taken at the level at which the gynoecium becomes disjoined from the perianth tube, shows that three vascular bundles have moved out to the periphery, leaving behind three remnants (placental strands) which lack xylem and soon disappear (Fig. 66). The bundles become the midribs of an outer, sterile carpel whorl. Alternating with the undifferentiated remnants are three other xylem-containing bundles which become the main cords of an inner, fertile whorl (Figs. 67–9).

In the ♂ flower the rudimentary ovary is unilocular and only becomes apparently three-chambered through the contact without fusion of three non-vascular protuberances which project into the ovary cavity opposite the three flat sides (Figs. 71, 72). This is the position occupied in the ♀ flower by the fertile carpels, which in the ♂ flower are not differentiated. Here, as in *Hydrocleis*, it is the absence of a differentiated inner carpel whorl in the ♂ flower which renders the gynoecium incapable of producing ovules.

Hydrocharis Morsus-ranae, L. *G* 6+6 (hitherto regarded as *G* 6) (Figs. 73–8). Here we have in a syngonous [1] flower the same scattered distribution of the ovules as in the more or less hypogynous types *Butomus* and *Hydrocleis* described above (pp. 472, 473), and an even closer approach to the *Papaver* construction owing to the projection of the fertile carpels into the ovary cavity in the form of incomplete partitions.

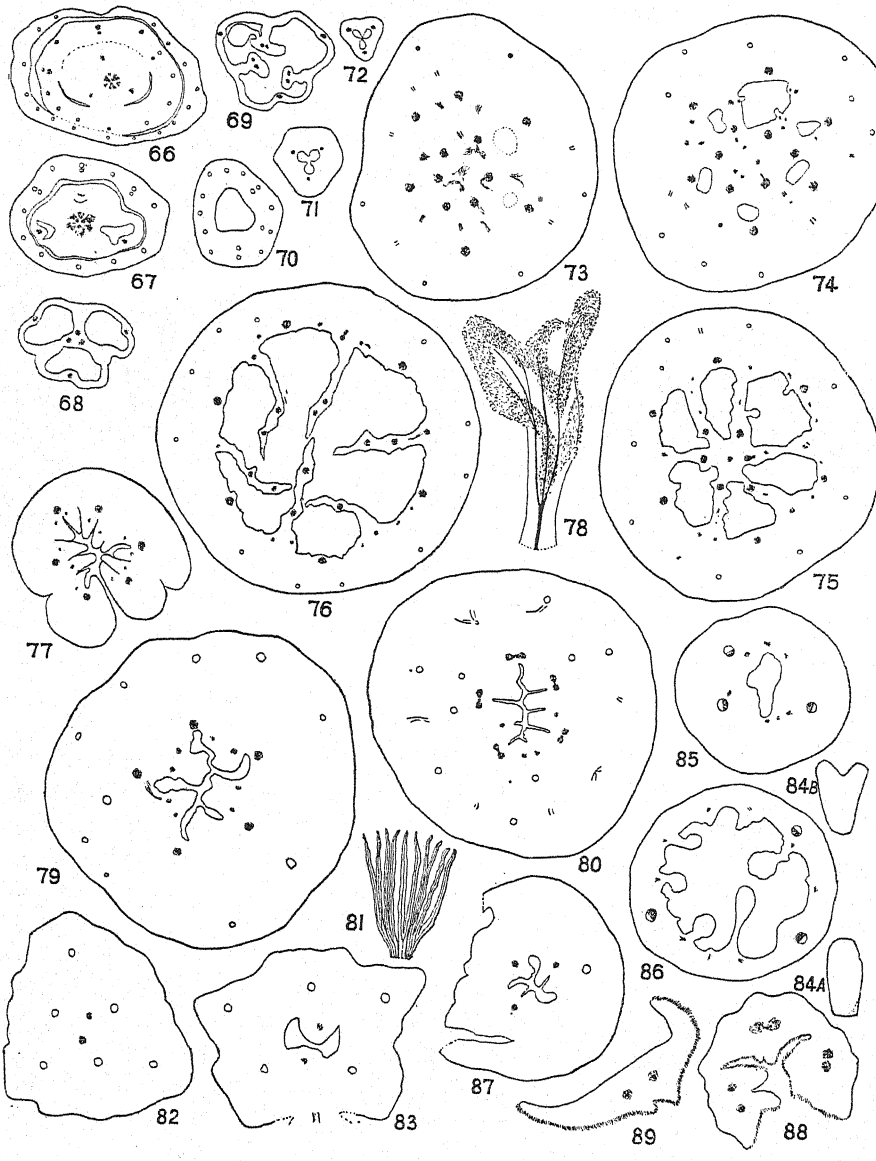
[1] See footnote 2, p. 470.

A transverse section through the base of the ♀ flower taken below the level of the loculi shows an outer ring of small bundles which supply the perianth. Within this outer ring is a second ring of six bundles on the radii on which the loculi make their appearance. These bundles become the midribs of the outer carpel whorl. Within these again is a third ring, also of six bundles, which lie on the alternate radii and from which strands pass outwards along the septa to supply the ovules (Figs. 73, 74). Some discarded strands may enter the pith, but these soon come to an end, and the pith, then clear, in turn soon ceases, so that the partitions (fertile carpels) become disjoined at their inner face, and the ovary becomes unilocular. The branches from the consolidated fertile carpels extend round the outer wall of the loculus, so that the ovules are distributed over the surface of the incomplete partition and of the outer wall. As the ovary becomes delimited at its apex from the perianth, the vascular tissue of each inner fertile carpel is reduced to twin bundles, the carpel splits radially between them, with the result that the six style filaments which are centred over the outer carpels, each represent a $\frac{1}{2}$ I $\frac{1}{2}$ carpel combination (Fig. 77). Each filament becomes strongly bilobed at the tip with occasionally a smaller projection between the lobes, and shows three vascular cords, corresponding to the one whole and the two half carpels (Fig. 78).

Stratiotes aloides, L. shows the same general arrangement on a more massive scale but its stigmas more closely approach those of *Trianea*.

Trianea bogotensis, Karst. (*Hydromystria stolonifera*, G. F. W. Mey) (Figs. 79–83). The ovary shows the same plan of construction as in the two preceding types, but in the ♀ flower the fertile carpels project less far into the cavity. The six stigmas differ from those of *Hydrocharis*, in that they are divided almost to the base, the two halves showing each a single vascular strand. These twin strands are derived from the splitting of the sterile carpel cord, for here the fertile carpel vascular system comes to an end at the ovary apex. One pair of these strands is therefore the counterpart of the undivided median strand in *Hydrocharis*, and the two corresponding half stigmas together represent one whole carpel. A rudimentary gynoecium is present in the ♂ flowers, but is inevitably barren, for only carpels of the outer sterile whorl are present, the style filaments being here undivided, and having each an undivided vascular cord. The full number of carpels in the ♀ flower is $6+6$, but in the ♀ specimen figured $G = 5+5$ (Figs. 79, 80), and in the ♂ flower $G = 2+0$ (Figs. 82, 83).

Vallisneria spiralis, L. (Figs. 84–9). Here reduction from the full monocotyledon type has taken the form in the ♀ flower of complete loss of vascular tissue on the alternosepalous radii, hence the members on these radii have either disappeared altogether, or they appear as non-vascular structures. We find $K\,3$ (sepals with midrib) $C\,3$ (or 2, as the result, at least in some cases, of partial or complete fusion between two members


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Figs. 66–89. All from transverse sections taken at successively higher levels except 78, 81, and 84. 66–72. *Blyxa flava* (?). 66–9. From a ♀ flower. 66. Flower base. On the outside the sheathing leaf (spathe), within which is the perianth tube still continuous with the spathe tissue to the outside and with the ovary on the inside. The three vascular bundles for the three stamens present have already been detached from three of the perianth cords. It is noticeable that the three stamens all stand on one side of the flower. In the ovary the cords of the three sterile outer carpels have already turned out towards the periphery, leaving behind in the centre a small strand of undifferentiated elements. On the alternate radii are three xylem-containing cords completing the central ring and destined for the three inner fertile carpels. 67. The same, without the spathe. The loculi have made their appearance in the ovary, which is now disjoined from the perianth tube. 68. The ovary below the ovule-bearing region and still trilocular. 69. The same, from the ovule-bearing region, now unilocular. 70–2. From a ♂ flower. 70. Perianth tube with the vascular strands for three stamens already formed. 71. The ovary, unilocular and infertile owing to the absence of the three inner carpels. 72. Apex of the same. 73–8. *Hydrocharis Morsus-ranae*, L., ♀ flower. 73. Flower base. The several whorls being still undifferentiated, the whole structure is solid. The vascular system serving the perianth ramifies in the outer zone (shown as very small dots in this and the following figures). Nearer the centre is seen a ring of six cords which supply the outer sterile carpels. Within this ring are six alternate cords together with numerous strands irregularly distributed and running in different directions. This inner system is destined for the inner carpel whorl. The loculi are beginning to be defined. 74. The conjoined perianth and gynoecium. At the outer border of each loculus is the cord of the corresponding sterile carpel. On the alternate radii the vascular system of the fertile carpels is still not yet fully differentiated. 75. The same. The fertile carpels are now seen as radial partitions continuous on their inner face with the pith. Lateral strands extend outwards from the central midrib cords. A last remnant of discarded vascular tissue is seen in the centre. 76. The same. Through the disappearance of the pith the fertile carpels now form incomplete partitions and the ovary becomes unilocular. 77. Ovary apex becoming free from the outer whorls. The central cavity now appears 12-rayed; six of these channels represent the contracted loculi; the other six are formed by a median radial split extending from the centre of each fertile carpel outwards so that these carpels are eventually split in half (shown in one case). 78. One of the six style filaments composed of $\frac{1}{2}$1$\frac{1}{2}$ carpels, the midrib of the sterile carpel in the centre, and on either side half the main cord of the neighbouring fertile carpel. 79–82. *Trianea bogotensis*, Karst. 79–81. From an exceptional ♀ flower with a pentamerous gynoecium. 79–80. Stages intermediate between those shown for *Hydrocharis* in 76 and 77. The twin bundles of the fertile carpels are gradually coming to an end. 81. The five stigmas each split almost to the base, showing a single vascular strand in each limb. 82, 83. From a ♂ flower. 82. The androecium of six stamens; in the centre the vascular cords for two carpels. 83. The same. The two carpels are now disjoined from each other, though still conjoined with the stamen ring. 84–9. *Vallisneria spiralis*, L. ♀. 84 A and B. The two members of the non-vascular corolla from a flower in which one member consisted of a single petal, and the other of two petals joined together. 85. Flower base, showing the three compound vascular cords destined for the sepals and carpels; around the loculus the strands which run to the ovules. 86. The ovule-bearing region of the flower. The ovules are borne at scattered points. 87. The ovary now disjoined from the calyx on the left but still conjoined with one of the sepals on the right. 88. Ovary apex now wholly free from the perianth. The three carpels are becoming disjoined from each other to form the three short styles. 89. One of the three stigmas, showing only a slight median indentation in the outline but complete divisions of the midrib into twin bundles.

which for want of space have been pushed out of their strict position and towards one another), without vascular tissue (Fig. 84), *G* 3, consolidated, in line with the sepals.

A cross section through the base of the syngonous[1] flower after it has become disjoined from the ensheathing spathe, but while perianth and gynoecium still form a solid cylindrical structure, shows three strong peripheral vascular cords. These composite cords represent the conjoined midribs of the three sepals and three semi-solid carpels. Lateral veins from these cords extend right and left along the border of the single loculus, giving off strands at various points to the ovules, hence the appearance of 'superficial' placentation. At the level at which the sepals are exserted and the ovary becomes free, the consolidated carpels begin to show the very characteristic radial split extending from the loculus border outwards in line with the midrib, and at the same time the midrib bundle divides in two. But this splitting of the carpel is never complete, and with the opening of the now 3-rayed loculus on to the surface the three members become disjoined, each whole carpel terminating in a broad flattened stigma having twin vascular bundles as in *Trianea*. It appears, then, that with suppression of the inner carpel whorl the outer carpels have become expanded laterally—they have become semi-solid. Further, they have not only taken on the form and function of the inner carpels but, like these carpels in *Hydrocharis*, *Stratiotes*, and *Trianea*, they bear numerous rows of ovules.

Summary.

The considerations set forth in the above account lead to the following conclusions:

1. That in Potamogetonaceae, Juncaginaceae, and Alismataceae, only one type of carpel is present, all the carpels whether few or many being of valve form. It follows that the currently accepted formula for the gynoecium remains unchanged.

2. That in Typhaceae, Sparganiaceae, Aponogetonaceae, Butomaceae, and Hydrocharitaceae the carpels are of two types, one of which is sterile, the other, fertile. Hence the usual formula for the gynoecium requires to be amended to bring it into accord with this polymorphic condition.

3. That in Typhaceae and Sparganiaceae the evidence clearly indicates a bicarpellary construction for the normal gynoecium. The contradiction inherent in the current conception of a solitary, terminal carpel is thus removed.

4. That the occurrence in *Sparganium* of single two-styled and of 'double' two-styled ovaries is due to bifurcation of the axis, occurring once in the former, and more than once in the latter case.

[1] See footnote 2, p. 470.

5. That the form of placentation occurring in Butomaceae and some Hydrocharitaceae described as 'superficial', and regarded as anomalous, is merely an extended form of the ordinary scheme of distribution of the ovules normal to the consolidated carpel type—a distribution which is seen among Dicotyledon types in *Papaver*.

6. That the infertility of the ♂ flowers of *Hydroeleis*, *Blyxa*, *Trianea* and, no doubt, of many other diclinous forms is due to the fact that although the outer, sterile whorl of carpels is present, the inner, fertile whorl does not take shape.

In conclusion, I desire to express my very grateful thanks to Miss D. F. M. Pertz who made the drawings here reproduced.

CORRIGENDUM

On Carpel Polymorphism, ii. Ann. Bot., xli, p. 596, Fig. 124.
For *vsb* read *wb*.





The Structure of the Seed Coat in Gossypium and its Relation to the Growth and Nutrition of the Lint Hairs.

BY

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With five Figures in the Text.

THE structure of the seed-coat in *Gossypium* has been described in a general way by Bowman (1) and Flatters (2), and in more detail by Balls (3). All agree on its broad differentiation into palisade cells, mesophyll and epidermis. Balls, however, distinguishes the two integuments, then unaccountably describes the palisade cells as a radial growth of the outer epidermis of the inner integument. He also describes a curious outward movement of the nuclei of these cells in advance of a secondary thickening in which they become finally imprisoned.

With regard to the origin of the cotton hairs, Bowman describes them as more active sub-epidermal cells that push their way through the cuticular layer. Balls, Denham (4), and Flatters show that they are specialized epidermal cells, which begin their growth in length about the time of fertilization of the flower, and Balls asserts that the number of cells that take on this specialization is influenced by the nutrition of the ovule at this time.

The only description of the epidermal cells is that shown by a diagram in Strasburger's Text Book, p. 116, and reproduced by Bowman. Balls, however, states that they have a thick basal wall separating them from the sub-epidermal layer, with thinner side walls and a thin cuticle covering the outer wall and dipping between the side walls.

OBSERVATIONS.

The present writer prepared sections of the seed-coat of Egyptian cotton, using both fresh and embedded material.

The alternate hard and soft layers of the tissues present some difficulties in obtaining satisfactory preparations, especially of the mature seed, but the

more perfect fragments show up better than more complete but thicker preparations.

The drawings in Pl. I were made with the aid of a Leitz projection apparatus at a magnification of 300.

The preparations of the surface view of the epidermis were made by scraping the fresh seed-coat with a knife and mounting in glycerine. It is somewhat remarkable that the presence of stomata on the cotton seed-coat has not hitherto been observed, and the only reference found by the writer to their presence on any seeds is in Sach's Text Book of Botany, p. 103, where they are recorded on *Ricinus*.

It will be seen from drawings that two integuments show the typical leaf structures of epidermis, palisade and mesophyll with vascular tissue. The palisade tissue occurs in the normal position in both structures, i. e. on the outer side of the inner epidermis, or, in other words, beneath the epidermis of the upper surface of the leaf.

The palisade tissue of the inner integument does not develop after the second week, and along with the mesophyll becomes crushed and disorganized by the developing embryo, so that on maturity of the seed it is nothing more than a parchment-like membrane between the seed and its outer shell.

The palisade layer of the outer integument has a very different history. From the twelfth to the twenty-fourth day after fertilization it continues to elongate in a radial direction until it is about twelve diameters in length. Its cells fit very closely together from their inner ends up to about two-thirds of their length, at which point the cell walls bend inwards, forming a shoulder and leaving intercellular spaces. This bottle-neck shape of the outer portion is only visible in thin sections in which a clear view is possible through the intercellular spaces. In thicker sections the space above the shoulder is obscured by the appearance of the bottle-neck shape of the cells below. This gives rise to the optical illusion of a row of rounded bodies (nuclei!) in a line corresponding to the row of shoulders. This row appears to advance in an outward direction as the cells elongate, in accordance with the description given by Balls of the movements of the 'nuclei', *pace* his assurance on page 55 that 'all his observations were repeated sufficiently often as to leave no doubt as to their truth and certainty'.

On the completion of this growth in length of the palisade cells, secondary thickening begins from the base, and continues until the cells below this shoulder become one solid mass of woody tissue. The intercellular spaces between the outer portions of the palisade cells are continuous with those of the mesophyll and the cavity below the stomata.

The outer epidermis shows the differentiation of the lint hairs immediately after flowering. After three or four days the diameter of the hair is four times that of the basal portion attached to the epidermis. The neighbouring epidermal cells grow considerably in size in each direction, but

more particularly tangentially, so that the base of the hair cell is crushed between its expanding neighbours. In this way the base of the hair becomes pointed or flattened, according to whether the pressure develops at the

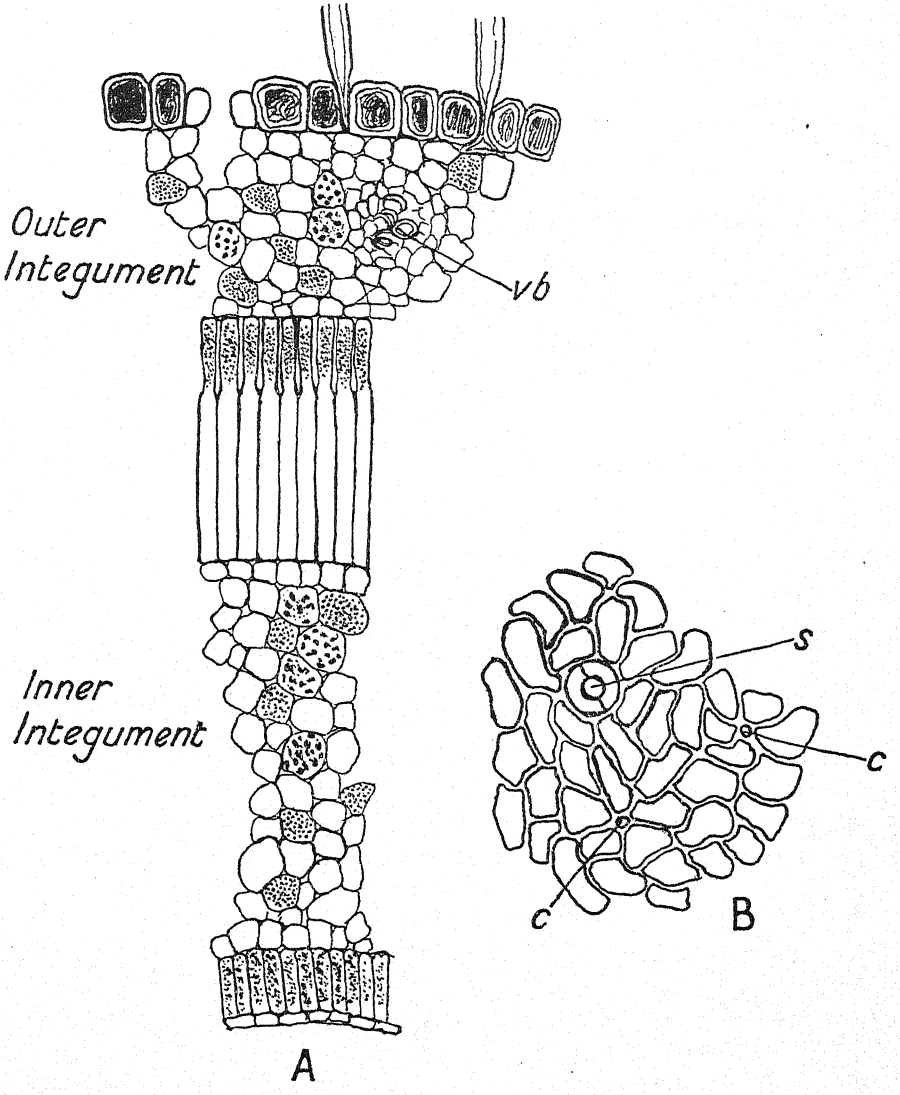


FIG. 1 A and B. Structure of seed-coat in *Gossypium*. A, in section, B, in surface view. *s.*, stoma; *c.*, base of cotton hair; *vb.*, vascular bundle. × 300.

lower or upper edge of neighbouring cells (Fig. 1 A and B). In the latter case the base of the cell appears expanded below the encroaching neighbours and gives some semblance of possibility to Bowman's idea of their sub-epidermal origin (Fig. 1 A and B). Viewed from the surface, the epidermal cells show the

usual undulating walls radiating from the stomata and from the base of the lint hairs. The dividing walls are indicated by canal-like depressions between the cells. The stomata show two semicircular guard cells with well-developed circular orifice, and are depressed below the surface.

The growth in size from the fourth day to the twenty-fourth day of the epidermal cells is about two-hundredfold, while that of the hair-cells is about sixteen-hundredfold. As the constriction of the base of the hairs

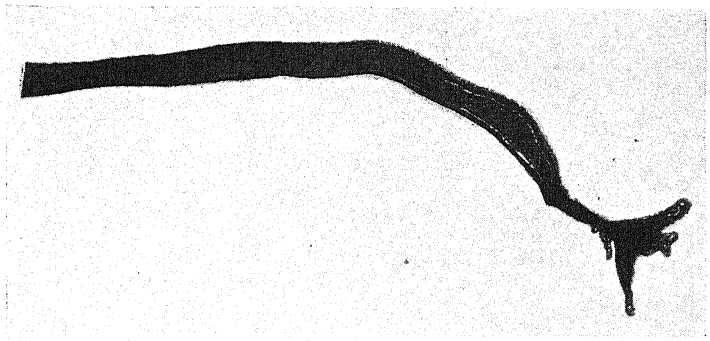


FIG. 2. Base of cotton hair with constriction due to lateral pressure of epidermal cells.

develops about the fifteenth day, the hairs become very easily detached from the seed-coat, and this condition persists until secondary thickening begins about the twenty-fourth day, after which the hairs become firmly attached to the seed-coat.

DISCUSSION.

The growth in length of the palisade cells until they become equal to twelve diameters and the close packing of the cells gives rise to difficulties in the mode of nutrition. The base of the cells is of small area and is not in contact with tissue suitable for translocation of assimilates. This difficulty is overcome by the development of intercellular spaces on the periphery of the cells connected with the vascular tissue of the mesophyll. The outer portion of the palisade thus resembles intestinal villi both in appearance and function, and provides a means for the rapid absorption of carbohydrates to meet the requirements of secondary thickening in these elongated cells.

During this growth of the palisade cells, the hair cells are also elongating rapidly, until they acquire a length of 2,000 diameters or more. As this growth involves movement of the cell-wall, it occurs in a liquid medium and its direction is probably controlled by nutrition in the same way as in the case of fungal hyphae (Fig. 3). This would account for the occurrence of branched hairs (6), as a response to environment rather than local pressure, as suggested by Denham. This liquid cannot be exuded by the

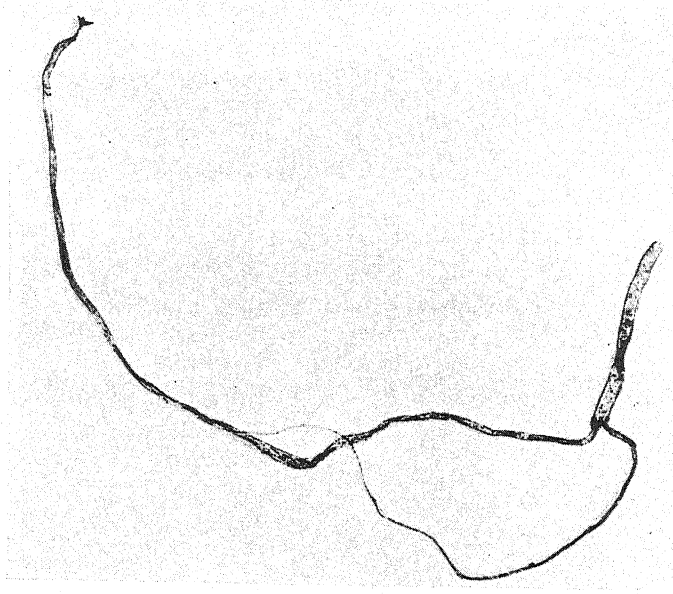


FIG. 3 *a*.

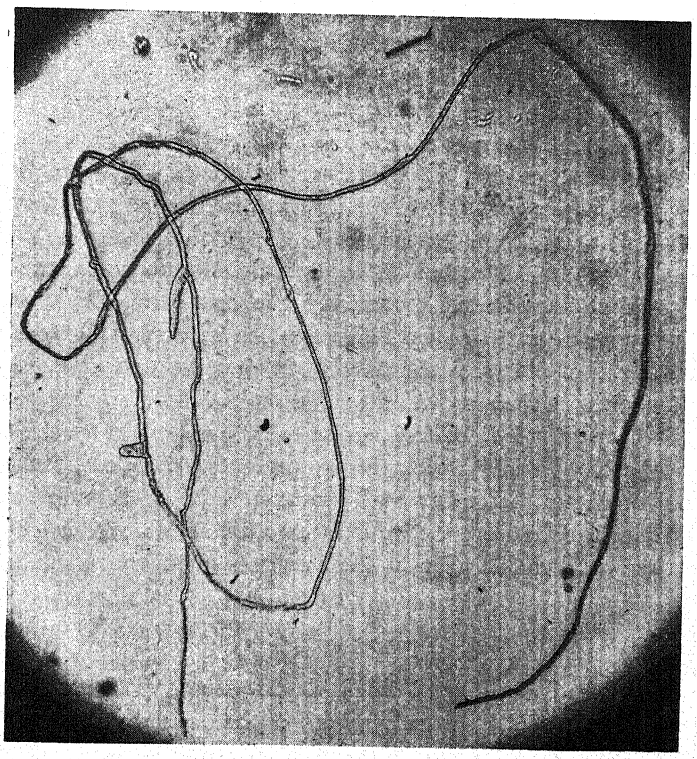


FIG. 3 *b*.

FIG. 3 A and B. Cotton hairs showing lateral branching which is probably a nutritive response.

epidermal cells, as they themselves are also in a rapid state of growth. Its presence is difficult to account for, unless we suppose that it comes through the stomata from the intercellular spaces of the mesophyll.

This liquid in the boll cavity disappears when secondary thickening begins, probably owing to the rapidity of the conversion of the sugar into cellulose. The apparent absence of free liquid from the boll cavity during secondary growth is not necessarily evidence that nutrition does not take place through the walls of the hair. A surface film of sugar solution is all that is necessary, in the same way that root hairs absorb adherent films of the soil solution from an apparently dry soil. If the growing hair absorbs food from the cavity of the boll before thickening, it is even more likely to do so afterwards.

Since thickening of the palisade layer, the epidermal cells and the cotton hairs all takes place simultaneously, there will be competition for the available assimilates coming from the vascular tissue, especially during times of diminished flow of sap. In this competition it is obvious that the hair cells will fare worst. The effect of this is reflected in the ginning out-turn of the crop and in the low hair weight per cm. of the lint. It is also obvious that the hairs will compete amongst themselves, and that fuzz hairs, being shorter and closer to the stomata, will have an advantage over the longer lint hairs. The fuzz hairs also begin secondary thickening at least ten days earlier than the lint hairs and thus are enabled to produce a much thicker cell-wall. In this connexion it is strange to remark that in Egypt a correlation is supposed to exist between the presence of fuzz and good quality cotton, and even forms the basis of seed selection among persons of technical training (7). Thin-walled cotton will certainly tend to have a low weight per cm., but will not necessarily make good yarn.

Further, Balls accounts for the rise in the ginning out-turn of the later pickings as due to the effect of an increased food supply at the time of flowering, which, he maintains, increases the number of hairs per seed. Such an influence of nutrition on differentiation seems hardly possible, but a more copious flow of sap to the tissues of the seed-coat would enable the lint hairs to continue their growth after the requirements of the competing tissues have been satisfied. Confirmation of this was found by the writer in the case of cotton plants which, being protected by glass, continued to mature bolls during December and January. The bolls took twenty to thirty days longer to mature, and the lint showed an increase in weight per cm. of 25 per cent. above that of the lint grown on the same plants in September.

Summary (5).

1. The structure of the seed-coat is described and criticism is made of the account given by Balls of the cytology of the palisade layer.

2. The occurrence of well-developed stomata on the seed-coat has not previously been recorded.[1]

3. No development of the base of the hair-cells occurs after differentiation. The neighbouring epidermal cells, however, develop enormously and subject the former to considerable lateral pressure which, together with thickening of the wall, closes the lumen at the base of the hairs at an early age.

4. The boll cavity is regarded as an intercellular space continuous with that of the mesophyll, and the nutrition of the lint hairs, like that of the palisade cells, is derived from the intercellular liquid.

5. The occurrence of branched hairs is explained as a response to physiological requirements, and not to local pressure.

6. The rise in the ginning out-turn in late autumn is due to the effect of nutrition, not on differentiation, but on the growth of the hair cells.

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[1] Since going to Press the writer learns from a private communication that Miss G. Clegg also made this observation at the Shirley Institute in 1926.

A Humariaceous Fungus parasitic on a Liverwort.

BY

E. J. H. CORNER, B.A.

With six Figures in the Text.

FEW discomycetes are known to be parasitic on bryophytes, though many live among such plants. The known instances belong almost entirely to inoperculate genera and do not appear of unusual construction. The fungus described in this paper, however, indicates a distinct class of bryophilous parasite, for it is an operculate discomycete with the vegetative characters of a mildew and fruit-bodies which resemble perithecia. Its connexion with the Humariaceae can be recognized, nevertheless, from the presence of carotin in the apothecium.

The fungus was recently discovered in France and is as yet unrecorded for this country or elsewhere. It is certainly overlooked through the smallness of the fruit-bodies, but it may be rare, for the writer has found it hitherto only in one part of a beech wood in the Chilterns, where the Liverwort abounds.

The specific diagnosis given in this paper is based on the writer's observations because the original seems inaccurate; the points of disagreement will be discussed in connexion with the systematic position of the fungus. The nomenclature which has been followed is that given by Boudier (1).

DESCRIPTION.

Neotiella Crozalsiana, Grelet, in Bull. Soc. Myc. Fr., 1925, t. xli, p. 83, Fig. 1.

Apothecia *pale yellow to bright orange-red, minute, perithecioid, strigose with white hairs*: globose at first, then conical with an apical pore, 300–500 × 250–400 μ; excipulum plectenchymatous, 65–85 μ thick, cells irregular 6–12 μ wide, composed of two layers, an inner of thin-walled hyphae containing carotin and an outer of thick-walled hyphae more or less devoid of carotin: hairs projecting at various angles from the surface cells, abundant, colourless, cylindrical or tapering to a blunt apex, straight or slightly flexuous unbranched, thick-walled with the lumen sometimes nearly

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obliterated, apex similar or thin-walled, septa few and thin, up to 300 μ long, mostly 100–150 × 10–6 μ at base, 5–7 μ at apex: *paraphyses* abundant, *filiform*, *attenuate*, flexuous, branched below, septate with the cells short and containing carotin below, longer and colourless above, 4–5·5 μ wide at base, 2–3 μ at apex.



FIG. 1. A portion of the Liverwort bearing apothecia, × 25, and two extreme forms of ascospore. × 1,000.

Asci 8-spored, fusiform with an oblique operculum, 170–200 × 27–30 μ (when spores are mature): iodine-ve.

Spores biseriate, elliptic to subcylindric, slightly inaequilateral, hyaline, *smooth*, *exospore thickened at each end*, usually *3-guttate*, median gutta large, pale orange, terminal guttae smaller and paler, with several colourless guttulae, 35–46 × 14–15·5 μ.

Mycelium *superficial*, sparse: hyphae colourless, thick-walled, cells long, 5–7·5 μ wide, *with cuspidate, ovoid appressoria*, 18–25 × 10–13 μ on short lateral hyphae, *and intracellular haustoria*.

Hab. ectoparasitic on *Plagiochila asplenioides*, (L.) Dum., Hampden Leaf Wood, nr. Wendover, Bucks, April–June, 1925, 1928.

Vegetative Characters. The hyphae lie close to the surface of the Liverwort. They do not grow into the apical meristem though they may be found between half-developed leaves, and they die off in older parts which have turned brown. At the edge of a leaf some grow into the air to an adjacent surface, but the majority curl round and spread on to the other side. The walls of the hyphae adpressed to the surface are unequally thickened, 2–3 μ thick on the exposed side and about 1 μ on the other, while they are uniformly 2–3 μ thick in the aerial hyphae: in some cases the lumen of the cell is nearly obliterated. The cells contain 2–5 nuclei,

possibly more, and are frequently connected with other hyphae by H-pieces, so that the mycelium is a true network.

The haustorial branches are specialized laterals which arise singly from the cells of the hyphae at varying intervals, rarely from consecutive cells; they consist of a stalk and a terminal appressorium. The apex of

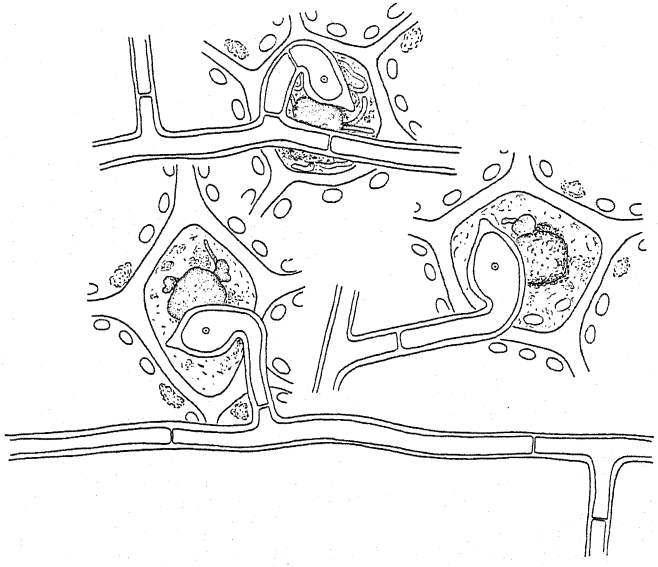


FIG. 2. Haustorial branches and the invaded host-cells in surface view. × 540.

the lateral is sharply pointed and first grows at a wide angle from the parent hypha, but soon turns backward, sometimes forward, though at rather more than a right angle, so that it is finally directed more or less toward the parent hypha; the inflexed portion then enlarges into the appressorium. The stalk varies in length, but is seldom more than 50 μ long; it may consist of a single cell expanded distally or there may be a short cell between the parent hypha and the terminal cell, which is then bodily transformed into the appressorium. The beak of the appressorium, which is 3–6 μ long, though in some cases rudimentary, is merely the unexpanded apex of the lateral from which the cell-cavity has receded owing to the thickening of the wall. The wall of the appressorium is thickened like that of the surface hyphae, but is much thicker, 3–5 μ, on the exposed side. The shape of the branch is peculiar, not unlike the head and neck of a goose, and in a position roughly corresponding to the eye on the lower side can be seen in surface view a small circle with a dot in the middle, which is the point where the haustorium has arisen.

The haustorium is an elaborate structure consisting of a stalk, a central body, and several hyphal processes. The stalk is a strand of cytoplasm, less

than 1 μ wide, which connects the central body with the appressorium through a rigid tube. The tube, which is 10–14 × 2–3 μ, evidently belongs to the host-cell, for it is continuous with the outer wall and stains in the same manner. Both tend to stain deeply with gentian violet, and in rather

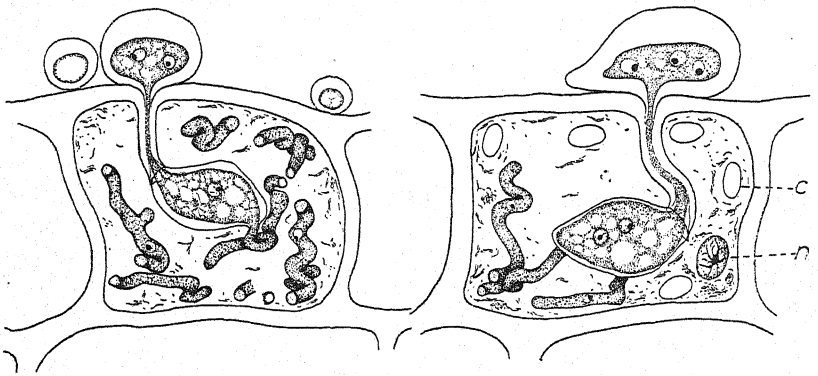


FIG. 3. Invaded host-cells, × 1,080: the appressorium is cut transversely in one case, longitudinally in the other; the fixative has made the cytoplasm in the central body contract. In one case the nucleus, *n.*, and chloroplasts, *c.*, are still intact.

overstained sections one can distinguish the boundary between the wall of the host-cell and that of the appressorium which counterstains with orange G. In such preparations the wall of the appressorium can be seen to become membranous on passing into the tube, and this appears the normal condition. Nevertheless, the stalk may have a distinct hyphal wall of its own and the tube may be very thin and even absent. Fig. 4 *a* shows a dead apparatus in which the stalk has the appearance of an ordinary hypha and there is no tube, whilst in *b* of the same figure the tube is very thick with no trace of a hyphal wall inside. It is possible that in the first case the tube had been broken off in the preparation of the section.

The central body is an ovoid turgid mass of dense protoplasm surrounded by a membrane which undoubtedly belongs to the fungus, for it can be traced into the opening of the tube, and is probably continuous with the membrane of the stalk. The central body stands more or less at a right angle from the stalk, often pointing obliquely upwards, and from its distal end one or two hyphal processes arise and coil around in the host-cell. The processes are 1·5–2 μ wide, colourless, aseptate, with dense contents and covered by the same membrane; it is doubtful if they branch.

The haustorium is not a distinct cell but a process from the appressorium. It is multinucleate, 1–3 nuclei occurring in the central body and 1–2 in the hyphal processes.

The way in which the haustorium penetrates the host-cell could not be determined, but, judging from the mature structure, it must be similar to that in the Erysiphaceae. In these fungi, according to Smith (11), a stylar

process grows from the lower side of a hypha and penetrates the wall of the host-cell. Apparently some enzyme, diffusing from the process in advance, swells the cellulose layer into a papilla, which is ultimately pierced at the apex and the process then expands into the body of the haustorium: the papilla thus remains as a short tube. In the present case

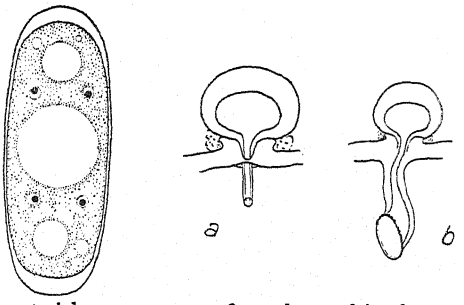


FIG. 4. Two dead haustorial processes, × 1,080, and a quadrinucleate ascospore. × 1,000.

the papilla must be much longer, and owing to the one-sided expansion of the process the tube has the shape of a ventilator. But microchemical tests for cellulose did not reveal any alteration of the cell-wall in this case, nor were the staining properties of the tube peculiar.

In some cases, as in *a* and *b*, Fig. 4, the wall of the host-cell round the point of penetration is everted and almost revolute, forming a circular ridge on which the appressorium is seated. The ridge may be caused by the swelling of the wall and the subsequent expansion of the stalk, but the matter is not clear.

It is possible that the hyphal processes ultimately pass into neighbouring cells, because a group of two or three dead cells containing narrow hyphae was occasionally seen round a single appressorium. However, there was no evidence of this in sections, and the narrow hyphae might have belonged to a mould which frequently grew into dead and unhealthy cells.

The haustorium soon destroys the contents of the host-cell and thus invaded cells, being colourless, are conspicuous in a healthy leaf. But the fungus cannot be considered a serious parasite. At most it may hasten the death of the older parts of infected shoots, and patches of the Liverwort sometimes have a ragged appearance for that reason. Usually there is no indication of the parasite in the growth of the Liverwort and the number of cells destroyed in a leaf is rarely as much as one quarter of the whole. Unfortunately no sporangia were found and it cannot be said, therefore, if the parasite affects the reproduction of its host.

Development of the Apothecium. Very few early stages of development were found and little can be said of how this proceeds. Practical difficulties in obtaining sections of such stages would be great, because the young apothecia are exceedingly small, widely scattered, and without

distinct orientation, owing to the tilt of the leaves. A certain amount can be made out with clearing agents and by teasing and crushing the tissues, which were the methods the writer used.

It seems fairly certain that the apothecium develops from a small knot of hyphae at the end of a short lateral projecting about 50 μ into the air.

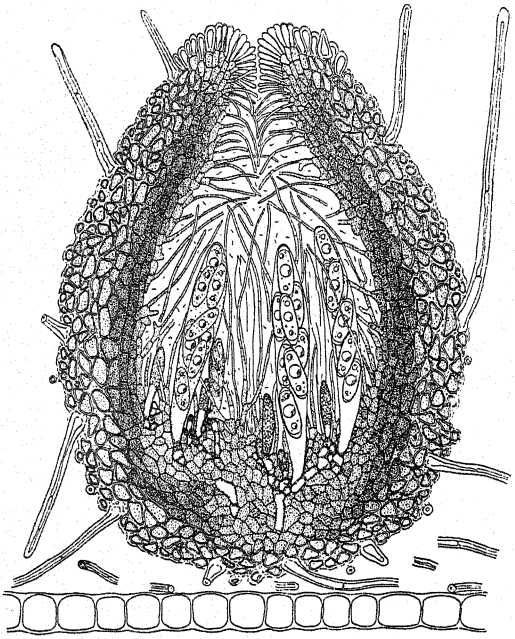


FIG. 5. Median section of an apothecium, × 250: the distribution of carotin in the sterile tissue is shown by stippling.

The knot enlarges into a more or less spherical body about 100 μ wide, more especially by the apical growth and branching of the internal hyphae. A small space then forms in the centre, owing to the dissolution of some internal cells, and numerous hyphae, 3–4 μ wide, grow into the space as the paraphyses. They enlarge the space into the mucilage cavity of the mature apothecium by addition of new paraphyses from their basal parts and by the gelatinization of the walls of their distal cells. Subsequently the layer overlying the mucilage cavity is ruptured, but by what means could not be discovered; the disorganization of the internal cells may extend to the upper surface or the layer may be ruptured directly by the pressure in the enlarging cavity. But in any case new hyphae grow out from the margin of the layer for a short distance, converging upwards, and on cessation of growth they leave a small pore surrounded by a definite margin at the apex of the whole structure. Paraphyses are also produced from these hyphae as laterals to the inside, but they are short and almost unbranched.

From almost the earliest stages hyphae grow out from the external cells of the apothecium, forming secondary mycelial connexions with the host in the lower part, but being of limited growth in the upper part they form the hairs.

No ascogonium was seen, which might have been due to the unsatisfactory method of examination: nor could the origin of the ascogenous

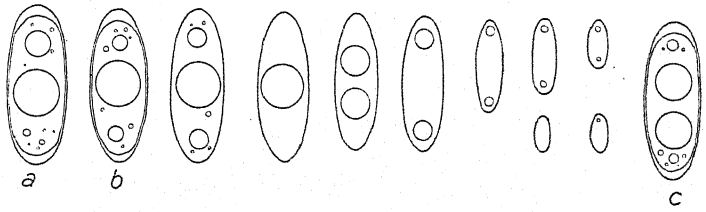


FIG. 6. Development of guttae in the ascospore, × 540: *b*, a normal mature spore; *a* and *c*, frequent variations.

hyphae be made out. But while the excipulum and mucilage cavity are enlarging the ascogenous hyphae ramify between the bases of the paraphyses in the lower part of the apothecium, which is thus bulged out, giving the conical shape to the mature structure. The asci grow up between the paraphyses and are directed at the pore.

The mature apothecium has been described as perithecioid in the diagnosis, and the description applies also to the development, which is characteristic of an angiocarpic apothecium, so far as it goes, but might equally well be that of a perithecium with short neck and ostiole. And, as will be shown, the description applies also to the mode of spore-discharge.

The excipulum is relatively well-developed. The distinction between the two layers is due to differences in the hyphal wall and in the abundance of carotin in the cells, in the form of small oily droplets. The hyphae of the outer layer have little pigment, maybe none at all in the outermost cells, and their walls are thick and cemented together in some firm mucilage-like substance probably derived from the walls themselves, whereas those of the inner layer contain the pigment in great abundance and their walls are much thinner and become diffluent next the mucilage-cavity. The difference in nature of the wall extends also to margin, where this is a regular layer of clavate cells, owing to arrest of growth; those on the outside are thick-walled, those on the inside thin-walled, as undeveloped hairs and paraphyses respectively. There are no air-spaces in any part of the apothecium, which, owing to the tough outer layer, is a remarkably firm body.

All the cells of the sterile hyphae in the apothecium are multinucleate, as in the mycelium. The colour of the apothecium is dependent on the amount of carotin.

Spore Development. The spores are delimited from the cytoplasm in the upper two-thirds of the young ascus and eventually fill the whole, except the extreme base. They are first visible in the living state as faint hyaline elliptical areas, $9 \times 5\,\mu$, surrounded by finely vacuolate cytoplasm. Shortly after delimitation a minute oil-drop appears at one end of each and is followed closely by another equally minute at the other end. The spore enlarges mainly in length and the oil-drops swell, acquire a pale orange colour, and when the spore has reached about two-thirds of its full length the oil-drops approach each other and fuse, thus forming the large median gutta of the mature spore. Two more minute oil-drops then appear, again one at each end: they enlarge, but not to the same extent as the first pair, become pale orange and persist without fusion as the two smaller guttae of the mature spore. Several other minute drops may appear towards the ends or near the middle about the same time as the second pair, but they scarcely enlarge and remain as colourless guttulae (Fig. 6).

That the guttae were oil-drops was shown by their solubility in fat-solvents and potash and by their staining reactions to osmic acid, &c.

The spores acquire their complement of oil-drops and reach full size before the final process of thickening of the exospore begins. The thickening takes place to a small extent all round, but especially at each end, and thus are formed the characteristic apical 'caps' of the mature spore.

A cytological investigation was not undertaken, but it was found that the spores, at first uninucleate, have 4–6 and possibly 8 nuclei at maturity. The material was insufficient to determine if nuclear division and gutta-formation were connected.

Variations on this mode of development are frequent. The first pair of oil-drops may not fuse, so that the mature spore has two fairly large median guttae, or one or both of the second pair may scarcely enlarge and the guttulae may be absent. Apical thickenings of the exospore are always present, but sometimes weakly developed.

Spore Discharge. The manner in which spores are discharged is fairly evident, though actual discharge was not observed. Mature fruit-bodies, which had been crushed in a drop of water were found to have contained a few asci, rarely as many as a dozen, with mature spores. But the asci were never seen under such conditions to elongate and dehisce, as commonly occurs in other discomycetes. Instead, after a minute or so, many, mature and immature, burst open irregularly, occasionally at the tip, and expelled their contents in an abnormal manner. Similarly the expelled spores, if immature and without a thickened exospore, burst, suggesting that the mature exospore is able to prevent excessive endosmosis.

But that an energetic dehiscence does ordinarily take place was shown by collecting the spores on a coverslip suspended over the fruit bodies. It was found that contingents of eight spores arrived on the coverslip at vary-

ing intervals of time, which meant that the asci had dehisced singly. The procedure must have been the same as in those Pyrenomycetes in which each ascus moves at maturity to the opening of the perithecium. If there was no elongation of the ascus into the pore in this fungus also, it is difficult to see how the spores could ever clear the opening and all eight be discharged together; and ascospores were never found free in the mucilage-cavity.

By the same method it was found that the spores could be shot to a height of 3·5 cm., in a saturated atmosphere at *ca.* 20° C. Whether this is a maximum cannot be said, owing to difficulty in directing fruit-bodies at the coverslip at greater distances. Negative results were obtained at 4 cm., but the supply of material ended before a conclusion could be reached.

Spore Germination. Many attempts were made to germinate the spores in tap-water, distilled water, in a moist atmosphere, and on various kinds of agar (Dox's, asparagin, and potato) at *ca.* 20° C.: but all failed. The contents of the spores may disintegrate after a few days or not until a week or so has lapsed; the guttae frequently diminish in size, and in a few cases only a remnant of the large gutta was left. In no case was there a sign of a germ-tube.

Spores were also transferred by a needle on to the Liverwort and left for a few days in a moist atmosphere, but they never germinated. However, among many spores that were seen lying on the leaves, three were found which had produced germ-tubes. In two of these a thin-walled aseptate unbranched germ-tube, about 70×4–$4{\cdot}5\,\mu$, had grown from the side of the spore near one end. In the third the germ-tube was $100\,\mu$ long with two septa, one near the spore and the other about half-way along the tube. From the cell next the spore a young haustorial branch had grown out, but had not as yet produced a haustorium. In all three spores some alterations in the guttae had occurred, but they were reduced in size and number beyond recognition.

It was thought from these failures that a period of dormancy might be required for the spore to mature. Accordingly spores were collected on coverslips and kept for 3–4 months at *ca.* 20° C., after which they were set to germinate, some in tap-water and some in distilled water. But in every case, six in all, it was obvious that the spores were dead and had not survived the desiccation; no germ-tube was produced.

Another negative point which may be mentioned here is that no subsidiary method of reproduction was found, so that these diffident ascospores provide the sole means of dispersal.

Systematic Position.

The fungus just described is certainly the species meant by Grelet; the spores and the habit are characteristic. But there are discrepancies between the two accounts. Firstly, Grelet describes the apothecia as 'primitus subglobosa, demum applanata' and rather wider, by about 100 μ, than what the writer found, but it is unlikely that so small a difference should signify an exposed hymenium and consequent alteration in fruit-body mechanism. One can, moreover, easily be misled if median sections of living apothecia are placed in water, because the two sides, being unconnected, are pressed apart by the swelling of the mucilage in the central cavity, giving the appearance of a small lenticular apothecium.

Secondly, Grelet's spore-measurements, 27–35 × 10–15 μ, are much smaller than the writer's, and if really taken from mature spores imply an unusual latitude in this respect. The spores themselves are described as 'intus ad extremitates vacuatae', which is without doubt an error. The exospore is thickened at each end and there is no space between it and the endospore as one is led to suppose.

And, lastly, neither superficial mycelium nor haustoria are mentioned in the original description.

A great difficulty is encountered, however, in classifying *N. Crozalsiana* with other discomycetes. The operculate ascus precludes any possibility of confusion with bryophilous pyrenomycetes having orange perithecia, e. g. *Nectria* spp., but the characters of the apothecium and mycelium are so distinct that it might seem advisable to create a new genus. But that is impracticable, for a new genus would require a new family and this again some higher rank. The difficulty is due to the absence of a system of classification which is natural to any great extent. Indeed, once the importance of the mode of dehiscence of the ascus is recognized, there remains but one system, that of Boudier, which after the primary division into Operculeae and Inoperculeae becomes wholly unnatural. Sections, families, and even genera are based merely on the form of the apothecium, with the result that few groupings higher than specific rank are exclusively defined, and thus wanting an essential of a natural classification of existing organisms. In attempting to place *N. Crozalsiana* in Boudier's system one is faced with the task of deciding whether it has a cupular, lenticular, or immarginate apothecium, all of which assume an exposed hymenium. Fortunately the affinities of the fungus are not only clear enough to avoid the difficulty but to illuminate the classification of discomycetes.

N. Crozalsiana is one of a large number of Operculeae, grouped in several families, but so well marked by the presence of carotin in the sterile hyphae of the apothecium, more especially in the paraphyses, that in all likelihood they form a monophyletic series. The bulk of the series is con-

tained in the Humariaceae of Boudier, but it also extends into the Ascobolaceae in *Lasiobolus* and *Ramsbottomia*, for example, and the Pezizaceae in *Sarcoscypha*, *Peziza*, &c. It comprises stipitate, sessile, cupular, and lenticular forms of apothecium ranging from 10 cm. to 0·05 cm. wide, and on the grounds that spore-characters are a guide to affinity it is itself composed of several parallel series. Moreover, many of the species which would be included in this series are bryophilous, e.g. *Humaria*, *Peziza*, although the status of the mycelium is unknown in any case. However, owing to lack of knowledge, it must suffice merely to draw attention to the pigment in the paraphyses as possibly providing a basis of natural order.

Thus, for the present, regard cannot be held for phylogenetic classification and the species in question is conveniently retained in *Neotiella* with other humariaceous fungi.

As recognized by Grelet, *N. Crozalsiana* must be related to *N. Notarisii*, Sacc., described in 1863 by de Notaris as *Peziza ascoboloides*. In that species, according to Saccardo (8), the spores are only 25 μ long with a single yellow gutta, apparently without apical 'caps', and the asci are projecting. It lives 'inter muscos', and in connexion with the apothecium there is the signal remark 'ore nonnihil angustata', which may mean that it opens by a pore. Now, if the apothecia of *N. Notarisii* are perithecioid, it is possible that the projecting marginal cells were mistaken for the tips of emergent asci. Furthermore, if the spore-characters were taken from immature spores (cf. the uniguttate spore, Fig. 6), every distinction between the two species disappears. But there is another, *Aleuria Ricciae*, Cr., which, according to Pelé (6), has orange apothecia, less than 1 mm. wide, colourless septate hairs 200 × 7 μ, subclavate paraphyses 6 μ, asci 160 × 24 μ, and spores 28 × 14 μ, with a single large gutta which, presumably, is colourless; it fructifies on living and dead parts of *Riccia glauca* and on the surrounding soil. Corda has an illustration of the species (3) which shows distinctly a lenticular apothecium and a suspicion of apical caps in the spore. The fungus is obviously a parasite belonging to *Neotiella*, though Pelé, who found the fungus, ignores mycelial characters and refers it to *Humaria*. Now if the apothecia of *N. Notarisii* are lenticular, with exposed hymenium, the species is distinguishable from *A. Ricciae* only by the colour of the gutta in the spore. The situation is insoluble, and can be overcome only by the examination of these parasites in every aspect. *N. Crozalsiana* indicates a class of discomycete similar to the Erysiphaceae, for it is unlikely that a parasite with an elaborate equipment and such obvious affinity should stand alone, and the class may similarly include biological species distinguishable by most trivial characters, so that one should not impugn these species of *Neotiella*.

The explanation of the perithecioid form of apothecium is simple, for it develops along the ordinary angiocarpic lines. In the angiocarpic

apothecium, e.g. *Ascobolus stercorarius*, the hymenium is formed in a mucilage-cavity within a primary sheath of interwoven hyphae, 2–0·5 mm. wide: the part of the sheath overlying the hymenium is eventually ruptured and the apothecium extends to its full size by marginal and intercalary growth. The early stages resemble the mature stage in *N. Crozalsiana*, the apothecium of which can thus be regarded as a persistently juvenile form. Normally the growth of the hymenium, especially of the ascogenous hyphae, forces the sides of the apothecium apart exposing the disc, but in *N. Crozalsiana* the growth is very limited and cannot cope with the small amount of marginal growth, which, consequently, draws the sides of the apothecium together, leaving a small aperture through which the spores are discharged.

It seems to the writer that two external factors have been involved in the production of this apothecium. Firstly, the fungus is specialized to an extremely xerophytic environment, for the Liverwort has no efficient water-supply. Infected patches were frequently found dried up during the fructification of the fungus, and the fruit-bodies can evidently withstand considerable desiccation; if left to dry for 3–4 days on the Liverwort they will continue to spore on revival with water, but no experiments were made, owing to lack of material. The thickening of the hyphal walls, especially on the exposed side, and the enclosed hymenium can be considered as special xerophytic modifications. On loss of water from the fruit-body the enclosure of the hymenium must be complete, for the contraction of the tissues will automatically close the pore and thus the ascogenous hyphae and young asci, which are the delicate part of the mechanism, will be entirely surrounded by the outer resistant layer of the excipulum; sufficient moisture is probably retained thereby in the mucilage-cavity to last over ordinary periods of desiccation. In this respect it is all the more surprising that the ascospores should be unable to withstand prolonged desiccation.

Secondly, the fungus must be impoverished, and lack of food may be the primary cause in the arrested development of the apothecium. The number of host-cells invaded is relatively few, and in contrast with the ordinary mycelium the absorbing surface is localized in the haustoria, whence food-materials travel along the hyphae to the centres of fructification; and incidentally this process suggests a certain number of haustoria, or a minimum drainage area, per apothecium. The function of a fruit-body is to produce spores, and when the supply of food-material is markedly restricted one would expect a reduction of the sterile tissue in favour of the sporogenous, as in the minute apothecia of *Ascodesmis*, *Pyronema*, *Ascophanus*, &c. In the present case the xerophytic factor forbids the reduction of the primary sheath and accordingly the growth of the ascogenous hyphae is restricted. The failure of the apothecium of *N. Crozalsiana* to expose a disc and the biological consequences can thus be regarded as embodied in the special mode of life.

The lenticular apothecium of *A. Ricciae* is of interest in this connexion, for it displays a less juvenile form, even in the subclavate paraphyses, which in *N. Crozalsiana* are clearly immature, not having developed the pigmented 'head' so characteristic of the Humariaceae.

General Discussion.

Two other points of interest emerge from the study of this fungus. The development of the guttae in the ascospores shows that there must be a special centre of gutta-formation at each end of the spore. The constancy of the guttae in the spores of Operculeae is well known, but apparently has never been interpreted developmentally. A consideration of certain types of spore suggests, however, the same regularity in development of the guttae as in *N. Crozalsiana*. The commonest type is the biguttate, ellipsoid spore roughly 15–25 μ long, e.g. *Peziza*. A second type in the uniguttate spore which may be globose, *c.* 20 μ, e.g. *Lamprospora*, or ellipsoid, 20–30 μ, e.g. *Helvella*. A third and much less frequent type is the triguttate spore, 30–45 μ, as in *N. Crozalsiana*: these spores frequently have apical 'caps' or appendages, e.g. *Gyromitra*, *Discina*, and the guttae evidently develop as in *N. Crozalsiana*, for their number, size, and position is always described as varying in the expected manner. A fourth type is the eguttate spore, which varies from 5 μ long in *Thelebolus* to 80 μ in *Ptychoverpa*.

It seems to the writer that these types may be correlated by the assumption that the basal type is the biguttate spore, with two centres of gutta-formation. In larger spores the two guttae fuse, giving the ellipsoid uniguttate spore, and if larger still two additional guttae arise giving the triguttate spore. In the globose uniguttate spore the two guttae may fuse at an early stage or one centre may be suppressed. In the eguttate spore either both centres are suppressed or have never been present. In the development of the triguttate spore one can then see a repetition of its former states. *A. Ricciae* and *N. Notarisii* may thus be antecedent to *N. Crozalsiana* in this respect. Nevertheless complications are introduced by multiguttate spores, and uniguttate spores can obviously arise in several ways. The writer suggests, therefore, that the development of the guttae, not the mature state, should be given in specific descriptions.

The second point concerns the ascus-mechanism. The mechanism is normally one of discharge by turgor-pressure, but it may degenerate into a state of indehiscence, which is particularly noticeable in Pyrenomycetes and Tuberales. Clearly some measure of the ascus-mechanism is needed, and as a rough index one can take the height of spore discharge. Close comparison between species with different spores would require difficult corrections for size, shape, aggregation, temperature, &c. But the following figures for height of discharge, which the writer has collected for Discomycetes, are significant: 2–3 cm. for *Aleuria rêpanda* and 35 cm. for

Dasyobolus immersus (Buller, 2), 2–3 cm. for *Morchella esculenta*, *Gyromitra gigas*, and *Ptychoverpa bohemica* (Falk, 4), 6–8 cm. for *Cheilymenia pulcherrima* (Woronin, 12), 2–3 cm. for *Thelebolus stercoreus* (Ramlow, 7), zero in *Thecotheus Pelletieri* (Overton, 5), in which the spores are merely squeezed out of the ascus, and at least 3·5 cm. for *N. Crozalsiana*. If 2–3 cm. indicates a generalized mechanism, then a lower figure should indicate degeneration which may be due to some defect in the ascus-wall, low turgor-pressure, failure of dehiscence mechanism, &c., and probably with loss of heliotropism and similar properties; and a higher figure should indicate the enhancement of certain properties. Overton states that the ascus-opening is too small in *T. Pelletieri*.

The importance of the datum is shown by the Tuberales, for the mark of a 'Tuber' is a cupular apothecium with indehiscent ascus. *Sphaerosoma*, which has been placed in the Tuberales and is omitted from Boudier's classification (1), is generally considered a true discomycete. In consistency, spore-characters, and subterranean habit it is similar to the Tuberaceae, but the construction of the apothecium is exactly the opposite. It is often made the starting-point for evolutionary series in the Tuberales, but the writer cannot discover in any account of the genus whether the asci are dehiscent. According to Setchell (9), Seaver describes the asci of *S. echinulatum* as operculate with an amyloid substance in the wall, and an amyloid substance is said to occur in the allied *Ruhlandiella*, which suggests a dehiscent ascus. On the other hand, illustrations always show the tips of the asci well below the surface of hymenium and the number of spores in the ascus may be reduced, 6–8; both are characters of the Tuberaceae. The anomaly of *Sphaerosoma* would largely disappear if one knew the ascus-index.

A second example is seen in *Daleomyces Gardneri*, which Setchell considers intermediate between Pezizales and Tuberales (10). It is subterranean at first with a convolute hymenium, yet the asci appear in every way normal; there is no mention of their dehiscence. The species is suspiciously like the Sparrassis-abnormality of the discomycete *Galactinia proteana*.

If details of the ascus-mechanism, more especially the manner and height of spore-discharge, were to be included in specific descriptions, the pale cast of the herbarium would vanish from the systematy of Ascomycetes. It is significant that the primary division of Discomycetes should be founded on just such a character.

Summary.

The discomycete, *Neotiella Crozalsiana*, Grelet, discovered in France in 1924, is recorded for England. A specific diagnosis is given.

The fungus is ectoparasitic on *Plagiochila asplenioides* (L.), Dum.,

causing merely the destruction of relatively few cells. It has special haustorial branches and a fruit-body similar in structure and mechanism to a perithecium.

The fungus is considered to belong to an unrecognized humariaceous series in the Operculeae, characterized by the presence of carotin in the paraphyses; the series includes many bryophilous fungi, some of which may also be parasites. In Boudier's system (1907) the species must be placed in *Neotiella*.

The fruit-body is interpreted as a persistently juvenile form of apothecium consequent on depauperation and xerophily.

The development of guttae in ascospores is discussed. There are apparently two centres of gutta-formation in the ellipsoid operculate spore, one at each end; the successive formation of two pairs of guttae and the fusion of the members of the first pair gives the triguttate spore.

The need for some measure of the ascus-mechanism is shown. The height of spore discharge is suggested as a rough index.

In conclusion, the writer wishes to express his indebtedness to the Department of Scientific and Industrial Research, from which he received a Research Grant during the year in which this investigation was made in the Botany School, Cambridge, and to Mr. F. T. Brooks for his advice in the preparation of the paper.

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Studies in the Gramineae.[1]

VII. On Hordeum and Pariana, with Notes on 'Nepaul Barley'.

BY

AGNES ARBER, M.A., D.Sc.

With nine Figures in the Text.

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[1] For references to previous papers in this series, which represents the work carried out with the aid of a grant from the Dixon Fund of the University of London, see nos. 2–7 in the Literature cited, p. 532.

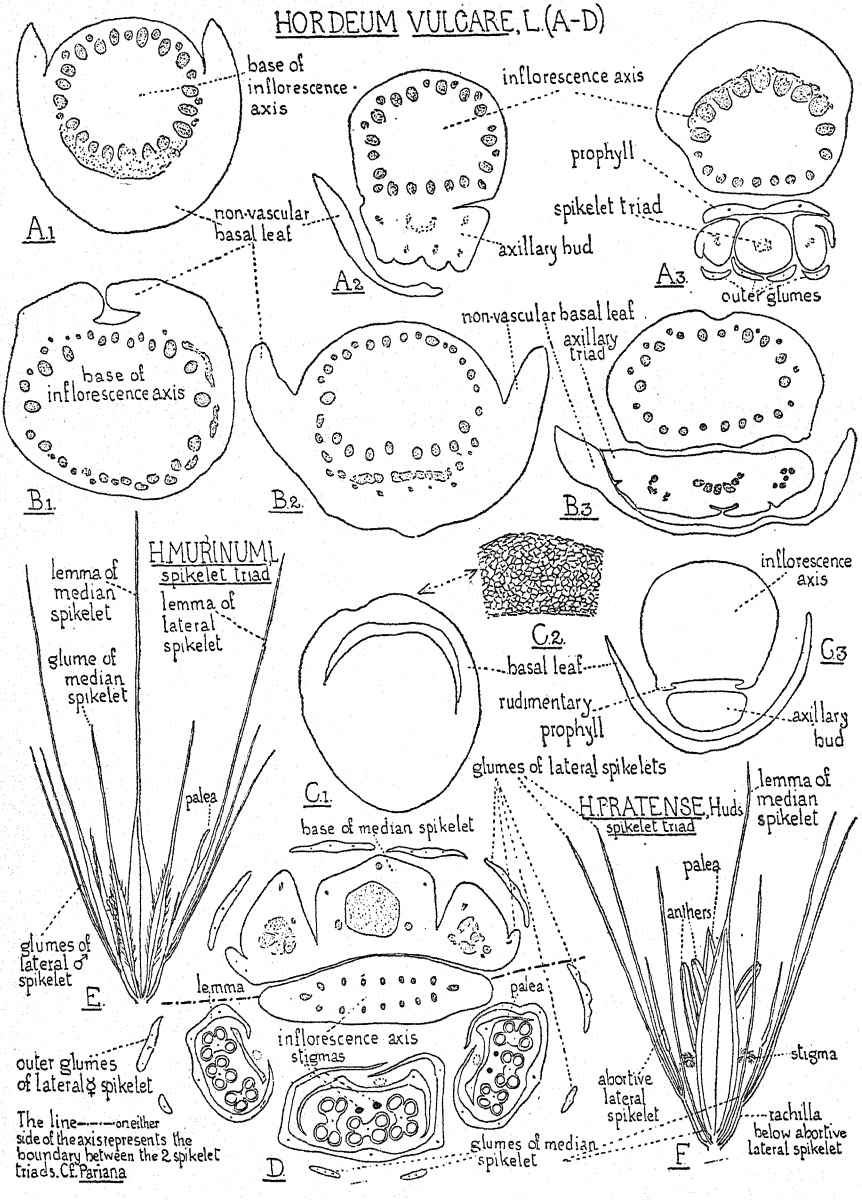


FIG. 1. A–D, *Hordeum vulgare*, L. A, B, C, series of transverse sections from below upwards through bases of inflorescences to show reduced non-vascular collar leaf (× 24). A, six-rowed Barley, Nat. Inst. Agric. Bot. Camb., 26.5.27, Ref. 774. B, six-rowed Barley, Garton's Squarehead Winter, Nat. Inst. Agric. Bot. Camb., 26.5.27, Ref. 831. B_3, from more than one section. C, Field Barley, Cambs., 26.5.27. In C_2 a small part of the free region of the leaf in C_1 is drawn (× 48) to show its parenchymatous structure. D, transverse section (× 24) passing through two triads of Garton's Squarehead Winter, Nat. Inst. Agric. Bot. Camb., 26.5.27, Ref. 831, to show general structure of a six-rowed Barley. All twelve glumes visible. Lower left-hand flower cut just above the top of stigmas. Rachillas of three flowers of lower triad dotted, as they do not reach

1. Introduction.

THE present study of spikelet structure in the genera *Hordeum* and *Pariana* was undertaken in order to see whether a comparative examination of the spikelets by the method of serial sections would throw any light on the nature of the problematic 'glumes', and also on the curious heritable abnormality known as 'Nepaul Barley'; in the course of the work other minor questions have come into consideration.

For material and other help I am indebted to Mr. R. A. Altson, Assistant Botanist and Mycologist, British Guiana; Mr. S. F. Armstrong, National Institute of Agricultural Botany, Cambridge; Mr. F. L. Engledow, School of Agriculture, Cambridge; Mr. Albert Howard, Institute of Plant Industry, Indore, Central India; Mr. R. J. Pearl, South-Eastern Agricultural College, Wye; Prof. A. C. Seward, F.R.S.; the Director and the Keeper of the Herbarium, Royal Botanic Gardens, Kew; the Director and the Superintendent, the Botanic Garden, Cambridge. I also wish to express my gratitude to the Council of Newnham College for the loan of laboratory equipment.

2. The 'Collar Leaf' of *Hordeum vulgare*, *L.*

The axis at the base of the ear in cultivated Barleys (*Hordeum vulgare*, *L.*) is surrounded by a ridge of tissue which we may call the collar. The appearance of this ridge suggests that it represents, in an extremely reduced form, the sheathing region of a leaf immediately preceding the inflorescence, and a study of the internal structure confirms this view. Figs. 1, A_1 and A_2, p. 508, show the collar in a six-rowed Barley; in this case it is detached obliquely, and the free upper margin is higher on one side than the other. Its identification as a leaf is borne out by the fact that it has a bud in its axil (A_2). This bud bears a prophyll on its adaxial face and develops into a triad of spikelets (A_3). The free part of the collar leaf is shown more clearly in B_3, which is an example from another strain of six-rowed Barley. Just above the level of B_3, the leaf, whose upper margin is irregular, dies out. Here again there is an axillary bud, which, however, bears no differentiated prophyll. In C_1–C_3 a third example from a different race of Field Barley is illustrated; the prophyll here is rudimentary and never becomes free from the main axis. I have also seen the collar leaf in a fourth race of Field Barley grown at the Cambridge Botanic Garden.

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to this level. The broken line on either side of the axis marks the boundary between the triads, cf. *Pariana*, Fig. 8, A_6, p. 529. E, *Hordeum murinum*, L. Triad of spikelets from abaxial side enlarged, but less so than F. Closed hermaphrodite central flower with fertilised ovary. Male lateral flowers more or less open. Anther emerging from male flower on left. F, *Hordeum pratense*, Huds. (*H. secalinum*, Schreb.). Triad of spikelets (×3 *circa*) from abaxial side. Clayhithe, 16.6.25. The fact that the glumes are scabrid is not shown. Central hermaphrodite flower open, showing anthers and stigmas. Lateral spikelets rudimentary.

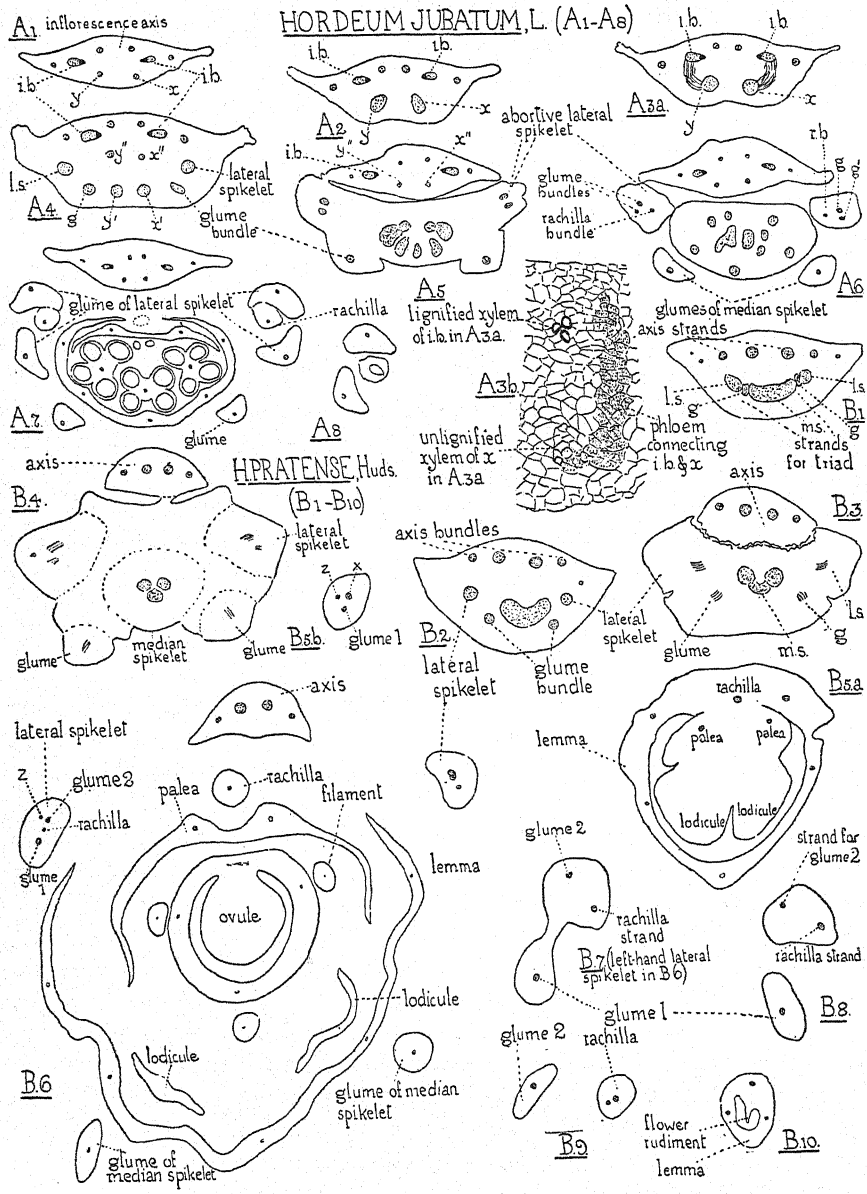


FIG. 2. A_1–A_8, *Hordeum jubatum*, L. Series of transverse sections (× 48) from below upwards through a segment of a young inflorescence, Camb. Bot. Gard., 28.6.27. A_1, axis below the production of a triad; *i.b.*, main inflorescence bundles; *x* and *y*, the bundles which will supply the triad and also replacement strands for the axis. For details see text, pp. 512–13; A_7, through anthers and stigmas of median spikelet; rachilla outline dotted, as it is visible at a slightly lower level. A_8 *b* shows the connexion of *x* and *i.b.* in A_8 *a*, or a similar section, on a larger scale (× 199), phloem dotted. The left-hand lateral spikelet cannot be followed above A_7, but A_8 represents a corresponding spikelet from another triad cut at a higher level, showing the rachilla hollowed into a leaf-like structure. B_1–B_{10}, *Hordeum pratense*, Huds. (*H. secalinum*, Schreb.) Clayhithe, 16.5.25. Series of

There would be no special reason for drawing attention to the basal collar leaf of *H. vulgare*, if it were not for one unusual feature, namely, that it is entirely non-vascular. I have recorded the existence of non-vascular leaves axillant to branches or spikelets in the inflorescence of *Luziola Spruceana*, Benth. (6, Fig. 1, B_4 and B_5, p. 392) and *Streptochaeta spicata*, Schrad. (7, Fig. 2, B and C_1, p. 38), but the non-vascular collar leaf of *Hordeum* is a much more striking object. I have counted as many as nineteen parenchymatous elements in the thickness of the free part (Fig. 1, C_2, p. 508). I do not know of any parallel among the flowering plants for a non-vascular leaf on such a scale.

3. The 'Glumes' of *Hordeum*.

(i) *Introduction.*

The typical *Hordeum* inflorescence consists of a flattened axis bearing two series of partial inflorescences (spikelet triads), one on either face (Fig. 1, D, p. 508). As a rule the spikelet triads have no axillant leaf and bear no prophyll, but in the basal region of the inflorescence these members are occasionally to be found, as we have noticed in the preceding paragraphs (Fig. 1, B_3, axillant leaf, A_3, prophyll, p. 508). Each fascicle or triad consists of a central spikelet flanked by two laterals, which are sometimes hermaphrodite (Fig. 1, D, p. 508), but may be male (Fig. 4, A_1–A_3, p. 517) or abortive (Fig. 2, A_7, p. 510, Fig. 3, A_6, p. 515). The spikelets show the ground-plan familiar in the Gramineae, except in one respect. At the base of the spikelet axis in normal Grasses, two leaf members occur, called 'outer empty glumes' or simply 'glumes'. They face one another; the lower arises on the same orthostichy as the flowering glume or lemma, while the second lies directly opposite. But in the Barleys, though two members occur below the lemma, their position is anomalous. Neither of them lies on the same orthostichy as the lemma; instead of facing one another, they both face the same way as the lemma, and lie more or less parallel to it, or forming various angles with it to right and left. These relations are indicated in Fig. 1, D, E, F, p. 508. The glumes are sometimes leafy (median spikelet in Fig. 1, E), but they may be bristle-like (Fig. 1, F). They thus diverge, both in situation and appearance, from the accepted idea of a glume. Their peculiarities have led certain botanists to the conclusion that they are not comparable with the outer empty glumes of the spikelets of other Grasses. Those who do not regard them as representing

transverse sections from below upwards through a segment of inflorescence, somewhat broken and reconstructed (× 48); *l.s.*, strands for lateral spikelets; *m.s.*, strands for median spikelets; *g.*, strands for glumes. B_5 *a*, median spikelet and its lemma alone; B_5 *b*, left-hand abortive spikelet only, at level of B_5 *a*. The bundle *x* in B_5 *b* gives rise in B_6 to bundles for rachilla and for *glume* 2. B_6 is drawn from several sections. B_7–B_{10} continue the history of the left-hand lateral spikelet; in B_9 *glume* 1 is omitted. B_{10}, rudiment of abortive flower. For details see text, p. 513.

these glumes have had to find some other interpretation. As long ago as 1848 Hochstetter (14), followed in 1883 by Körnicke (15) and in 1910 by Schuster (20), took the view that in *Hordeum* the second glume is absent, while the two structures present are to be regarded as the first glume split into halves. Martin Schenck (19), on the other hand, returned in 1907 to another view, also originally suggested by Hochstetter, that the 'glumes' of *Hordeum* are sterile spikelets. This view, as Schenck recognizes, implies the existence of nine spikelets instead of three in the *Hordeum* fascicle, since the pair of 'glumes' associated with each lateral spikelet must necessarily be explained on the same lines as those belonging to the median spikelet. Hackel (12), on the other hand, has maintained the view that the 'glumes' are rightly so named, and that they each represent one of the outer empty glumes of other Grasses; their displacement he attributes to the mechanical difficulty of fitting into a given space. Chase (8) has suggested that the abnormal position-relation between the glumes and lemma may be due to a torsion of the rachilla above the second glume.

From this brief *résumé* of the views that have been held, it seems clear that further facts are needed. In the following paragraphs I propose to analyse the results of studying the spikelet triads of various Hordeums by the method of serial sections, with special reference to the origin and relations of the 'glumes'. For descriptive purposes I shall now employ the word glume for these structures; whether its use is justified will be discussed on p. 518, on the basis of the observations detailed.

(ii) *Observations.*

(*a*) *Hordeum jubatum*, L.

It is convenient to begin the study of the *Hordeum* spikelet with the Squirrel-tail Grass, *H. jubatum*, L., since in this species the lateral spikelets are abortive and the general anatomical structure is relatively simple. Fig. 2, A_1, p. 510, shows the inflorescence axis below the level of origin of a triad of spikelets. The vascular skeleton is markedly dorsiventral. The two bundles, *i. b.*, occupying the foci of the section, are the most conspicuous objects. Two other bundles, marked *x* and *y*, are, however, the most important from our point of view. They will contribute a corresponding pair of bundles, *x''* and *y''*—replicas of themselves in position—for the axis above the next triad, and they are also responsible for the vascular supply of that triad; we may call them the 'fertile' bundles. In A_2 they have enlarged in preparation for division, and in A_3 *a* they have each put out a branch which comes in contact with the phloem of one of the principal inflorescence bundles, *i. b.* This connexion, which is shown in greater detail in Fig. A_3 *b*, does not last long—there is an almost immediate return to the condition of four discrete strands. A temporary bundle-connexion of this type

occurs below each triad, as I found by following the behaviour of the two principal inflorescence bundles over a segment of inflorescence including six successive triads. At the level of origin of the vascular supply of each triad, the inflorescence bundle was connected with the 'fertile' bundle by means of the phloem alone of the former. The two main inflorescence bundles were the only two lignified bundles in this segment of the inflorescence, and in none of the twelve anastomoses examined was there any sign of a connexion between the lignified xylem of the main bundle and the 'fertile' bundle. This seems to furnish some confirmation of a view expressed elsewhere (1, pp. 173–4) that the lignification of a bundle, and its efficiency as a conducting channel, have little to do with one another. For one can hardly suppose that these connexions, which form the only line of communication between the main bundles of the inflorescence axis and the spikelets, can be unimportant, and yet at this stage lignified xylem plays no part therein. It is true that the inflorescence is young, but the flowers are already differentiated.

In A_4, x and y have each given rise to three bundles for the spikelet triad. These six strands form an arc, the outermost member of which on either side (*l. s.*) is destined for the sterile lateral spikelet, while the next (*g*) is for the glume of the median spikelet. The two remaining bundles, x' and y', anastomose and branch, giving rise to the five central bundles in A_5, which have branched further in A_6. This group, derived from x and y, supplies the glume, palea, and flower of the median spikelet. In A_6 the spikelet triad is free from the inflorescence axis, while the median spikelet, its two glumes, and the lateral spikelets have all separated. The abortive lateral spikelets have each three bundles at this level—two destined for the glumes and the third for the rachilla. Fig. 8, A_7, shows the stigmas and anthers of the median spikelet, while the lateral spikelets have each given off two glumes. The abortive spikelet to the left in A_7 cannot be followed higher, but A_8 represents a similar spikelet from another triad, showing that at a higher level the rachilla hollows terminally into a rudimentary leaf (lemma), which does not, however, open out.

(*b*) *Hordeum pratense*, Huds. (*H. secalinum*, Schreb.).

For comparison with *H. jubatum* we may take the Meadow Barley, *H. pratense*, in which the vascular system is even slighter in construction. The external appearance of a spikelet triad is shown in Fig. 1, F, p. 508. Sections through the origin of a spikelet triad, showing the course of the bundles, are drawn in Fig. 2, B_1–B_{10}, p. 510. In B_1 the vascular supply for the spikelet triad is already differentiated as the zone to the south of the section, while the bundles for the continuation of the axis form the series to the north. The zone for the triad consists of five members: the two outer-

most, *l. s.*, are destined for the abortive lateral spikelets; the next, *g.*, for the glumes of the median spikelet; the middle member, *m. s.*, for the lemma, rachilla, palea, and flower of the median spikelet. These bundles are separating in B_2, and in B_3 the strands for the lateral spikelets and glumes are passing out, and the median strand is dividing into three; the process can be followed further in B_4, in which the spikelet triad is on the point of separating from the axis. In B_5 *a*, the median spikelet alone is represented, and in B_5 *b*, the left-hand lateral spikelet from the same level. It will be seen that this spikelet consists of an axis including three bundles; the one to the south is destined for the first glume; the bundle *x* divides to give rise to the bundle for *glume* 2 to the north and the rachilla bundle to the south; the bundle *z* has no further history, but disappears in passing up, apparently fusing with the adjacent strand. The entire spikelet triad and the axis are shown in B_6 at the level of attachment of the ovule in the median flower. In B_7–B_{10} the left-hand abortive spikelet in B_6 is traced to its apex. The detachment of *glume* 1 is seen in B_7 and B_8, and of *glume* 2 in B_9. The rudiment of the lemma and flower are reached in B_{10}.

(*c*) *Hordeum distichum*, L., var. *nigrum*.

As an example of a species showing a more massive vascular development in the inflorescence than *H. jubatum* or *H. pratense* we may take *H. distichum*, var. *nigrum*. The origin of the triad bundles follows the same general plan as that described for *H. jubatum*. The section of the inflorescence axis below a triad is shown in Fig. 3, A_1, p. 515; the four bundles to the south have enlarged in preparation for division. In A_2 they have fused with one another and with the two main inflorescence bundles. In A_3 the two main bundles have again become free, and three replacement bundles have been given off from the southerly series to fill the gap in the axis cylinder, while the four bundles to the north are passing out obliquely into the axis region. In the vascular zone to the south the elements run more or less horizontally and lignification is incomplete, so that xylem and phloem cannot be distinguished. From this zone a bundle is detached at the extreme left for the abortive lateral spikelet, and a bundle next within it for the median bundle of the left-hand glume of the median spikelet. A bundle lying in the cortex, whose origin I cannot trace, becomes the outer lateral of this glume. To the extreme right of the vascular zone the end bundle is correspondingly detached for the right-hand lateral spikelet, but the median bundle of the glume is in this case separated from that of the lateral spikelet by a small strand which forms the outer lateral bundle of the right-hand glume. In A_5 the detachment of these organs has gone further, while in A_6 it is completed, and the fertile median spikelet is cut through anthers and stigmas. Fig. 4, B, p. 517, which represents a flower

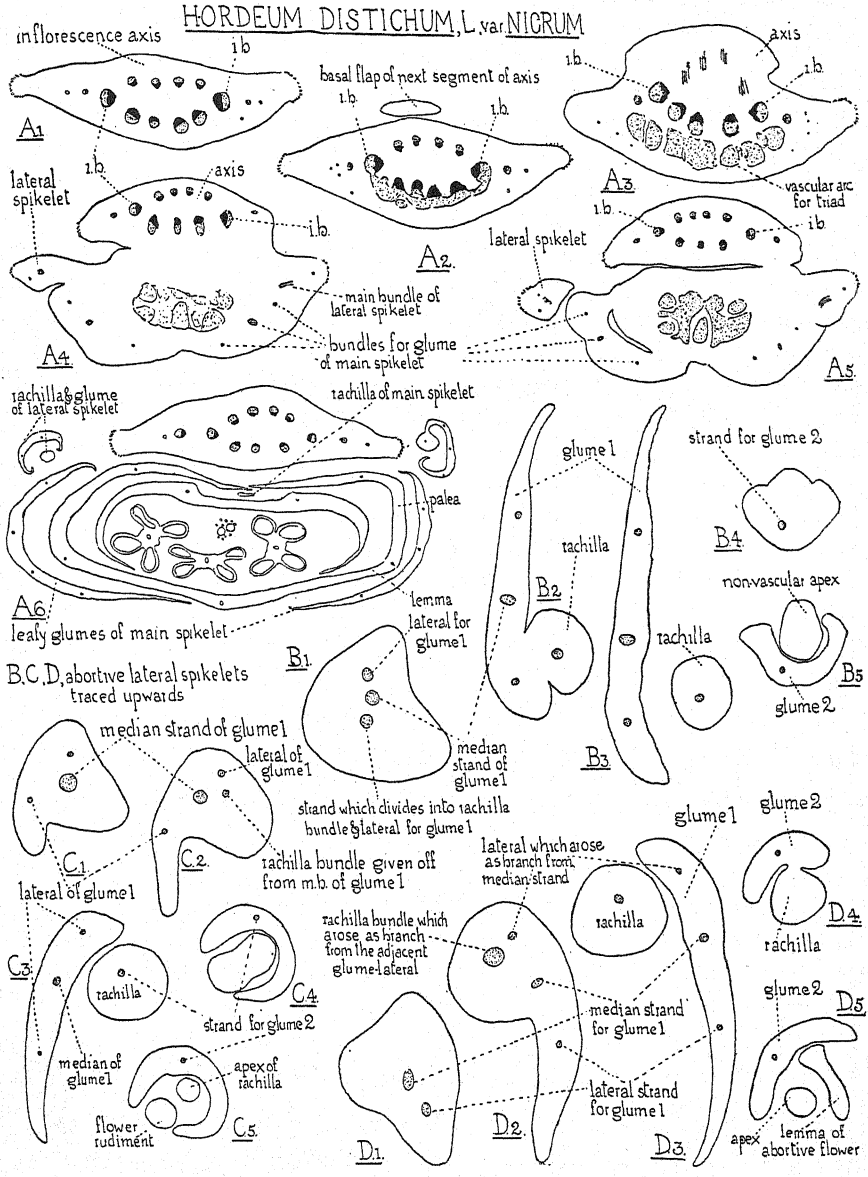


FIG. 3. *Hordeum distichum*, L. var. *nigrum*, Camb. Bot. Gard., 6.7.27. A_1–A_6, series of transverse sections from below upwards through a segment of an inflorescence, to show the origin of a spikelet triad (× 24); *i.b.*, main bundles of inflorescence axis. B, C, D, series of transverse sections from below upwards through three lateral abortive spikelets (× 80) similar to those drawn on a smaller scale in A_5 and A_6 to show the origin of the glumes and of the abortive flower. For details see text, pp. 514 and 515.

of this species cut at a somewhat lower level, is included in order to show the insertion of the ovule, which is on the adaxial wall of the ovary, as is the rule in the Gramineae. Schuster, however, has erroneously figured this attachment in *H. distichum* as though it were reversed (20, Fig. 4, p. 7).

Figs. 3, A_1–A_6, p. 515, are on too small a scale to allow the vascular history of the abortive lateral spikelets to be readily followed; it is better seen in B, C, D, in which three such spikelets, more highly magnified. can be traced upwards. B_1 shows the stalk of the spikelet already provided with three bundles. The central strand is not, as might be supposed, the axial bundle of the spikelet, but is the median bundle of the first glume; the strand to the north is the lateral for this glume, while that to the south will divide to form the rachilla bundle and the second lateral for the same glume. In B_2 and B_3 the glume is becoming free. In B_4 and B_5 we see the rachilla bundle passing into the second glume, and leaving the stem apex with no vascular tissue. C, another abortive spikelet, has three bundles from the base, representing the median and lateral bundles for *glume* 1. In C_2 a bundle for the rachilla has arisen, but in this case it comes off as a branch from the median bundle of *glume* 1 instead of from a lateral of this glume, as in B. In C_3 *glume* 1 is detached, and in C_4 the rachilla bundle has passed into *glume* 2. The non-vascular apex divides into two small cones, of which one is presumably the continuation of the rachilla and the other the flower rudiment. In the third example figured, D, there are two bundles at the base of the spikelet stalk (D_1)—the median and one lateral for the glume. The median gives off the second lateral to the north, and from this lateral the rachilla bundle arises. In D_3 the glume has separated, and in D_4 the rachilla bundle has passed into *glume* 2. The non-vascular apex divides into a terminal cone and a second rudiment which is probably the lemma of the abortive flower.

It will be seen that my observations on the origin of the glumes in the lateral spikelets are not in agreement with those of Schuster on *Hordeum distichum*; he figures them as both arising on the same orthostichy (20, Fig. 3, p. 217).

(*d*) *Hordeum murinum*, L., *H. bulbosum*, L., *and H. vulgare*, L.

In *Hordeum murinum*, L., *H. bulbosum*, L., and the strain of *H. vulgare*, L., of which a spikelet triad is illustrated in Fig. 4, A_1–A_3, p. 516, several bundles enter the bases of the lateral spikelets, which are male instead of being abortive as in the cases previously considered. Otherwise there is no essential difference in the vascular system and in the origin of the glumes between such forms and *H. distichum*, L., var. *nigrum*, described under (*c*).

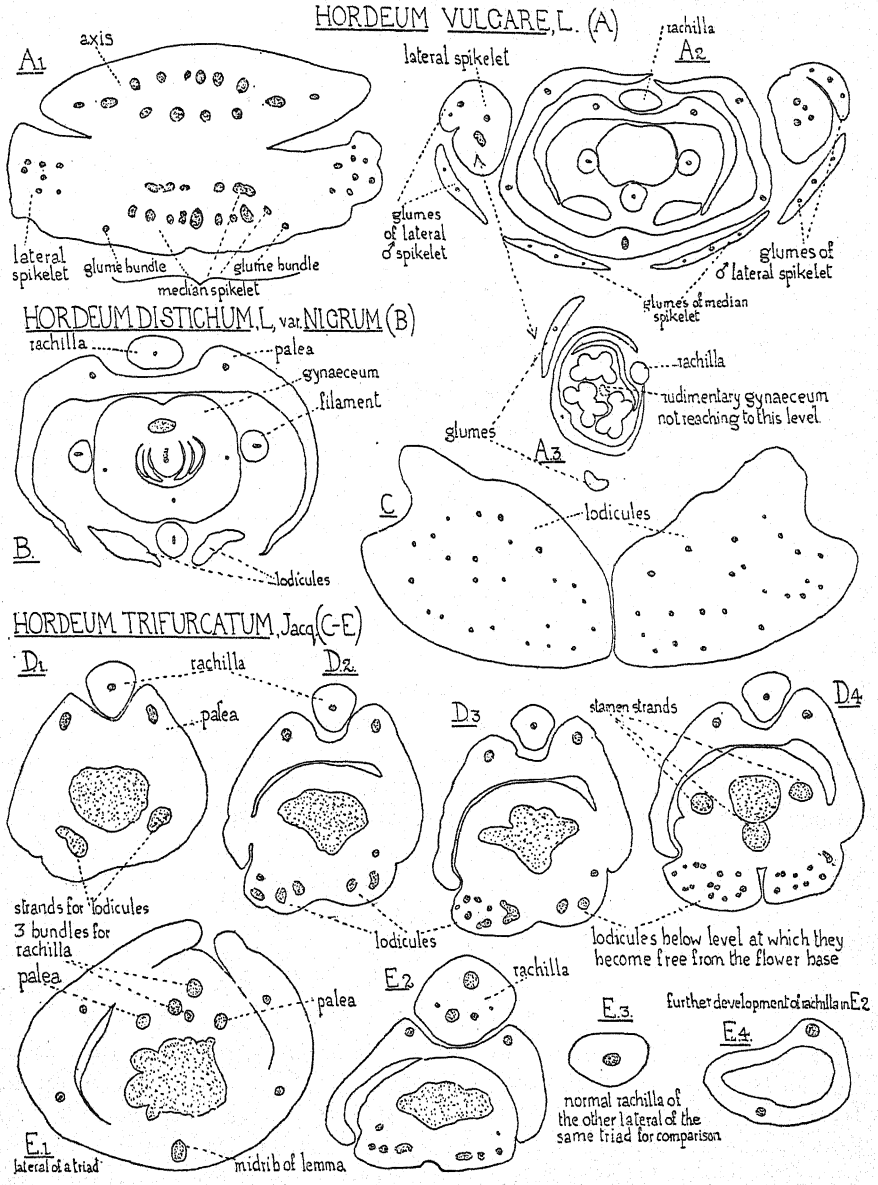


FIG. 4. A_1–A_3, *Hordeum vulgare*, L., transverse sections from a series through a young inflorescence of Field Barley, Cambs., 2.7.27 (same material as Fig. 1 C). B, transverse section of a spikelet of *Hordeum distichum*, L., var. *nigrum*, lemma omitted (× 48). C–E, *Hordeum trifurcatum*, Jacq.; C_1, transverse section of two lodicules from a flower (× 80). D_1–D_4, series of transverse sections from below upwards through the base of a flower from another spikelet to show vascular supply of lodicules (× 48). E_1, E_2, and E_4, series of transverse sections through a spikelet (× 48) to show an abnormal rachilla with several bundles which opens out above into a leaf-like structure (E_4). Lemma omitted in E_2. E_3, the normal one-bundled rachilla of the other lateral of the same triad (× 48).

(iii) *Discussion.*

(*a*) *The Nature of the 'Glumes'.*

We have now to consider the bearing of the observations detailed in the preceding paragraphs upon the question of the nature of the 'glumes' in *Hordeum*. As we showed on pp. 511, 512 three possibilities have been suggested. The 'glumes' may be:

1. Halves of the first glume, the second being absent.
2. Abortive spikelets.
3. True glumes equivalent to the outer empty glumes of other Grasses.

The first alternative has been, I think rightly, rejected by Hackel (11) on the ground that the glumes in their origin are separated from one another by nearly 180° of the axis. Reference to my figures will show that Hackel's objection, founded on external observation, is entirely borne out by the anatomy. The glume bundles, *g.*, in Fig. 2, A_4 and B_1, p. 510, for instance, are so widely separated in their origin that it would be fantastic to regard them as parts of one member. Nevertheless Schuster (20) states that the two glumes of *Hordeum* arise from one primordium. Like others who have used Payer's method of studying ontogeny he views the young inflorescence as a solid object, and deduces the relations of the embryonic members from the grades of relief which the leaf rudiments, &c., appear to present. But I much doubt whether any critical results in difficult cases can ever be obtained in this way, though it is possible to draw extremely pretty pictures of the appearances seen. It seems to me that the only way to get a rigorously exact idea of the spatial relations of embryonic organs is by the method of serial sections.

If we accept the second alternative—that the glumes are in reality abortive spikelets—though we are freed from the difficulties of the first alternative, we find ourselves at once involved in a series of complications. For if this theory be true of the median spikelet, it must also be true of the lateral spikelets which accompany them, and we are therefore compelled to interpret each of the six glumes associated with every spikelet triad (e.g. Fig. 4, A_2, p. 517) as being itself a spikelet. *Hordeum* would then be a genus in which outer empty glumes are entirely absent, which, though not impossible, would be surprising. But a more serious objection is that there is no one respect—external or anatomical—in which the 'glumes' show the characters of a shoot rather than a leaf. So the interpretation of the glumes as spikelets may, I think, be discarded as wholly improbable.

The third alternative—that the glumes are leaf structures equivalent to the two empty glumes of other Grasses—seems to me to have everything to recommend it. Their behaviour and anatomy are entirely foliar, and the

bristle-like form which they sometimes assume is merely a matter of reduction. The one point in which they diverge seriously from other outer empty glumes is their position in relation to the succeeding leaf—the lemma: in their situation they disregard the distichy normal for Grass spikelets. But for this difficulty I think that the serial sections which I have described offer a solution. In the first place these sections make it clear that there is no torsion between glumes and lemma such as has been suggested as a possible way of accounting for their abnormal position-relation. This explanation must thus be rejected. Moreover, the comparative anatomical study of the *Hordeum* inflorescence reveals the existence of a peculiarity which profoundly affects the whole structure and seems to hold the clue to our difficulty; this peculiarity is the *strong tendency to dorsiventrality* in the anatomy both of the inflorescence axis and of the bases of the triads. This is carried so far that the flattened axis may show an extremely flattened ellipse of bundles with two main strands to right and left (Fig. 2, A_1, p. 510) or even *a single series* of bundles (Fig. 2, B_6). In correlation with this special type of axis structure, the base of the triad, instead of being supplied by means of the radial arrangement of bundles which one expects to find in a shoot, is furnished with an extended arc or band of vascular tissue, whose arrangement recalls petiolar anatomy (Fig. 2, A_4, B_1, p. 510, and Fig. 3, A_3, p. 515). At higher levels the radial nature of the spikelet axes begins to assert itself, but at the extreme base, where the glumes originate, the dorsiventrality of the structure is so pronounced that the first members given off—the lateral spikelets and the glumes—have no alternative but to derive their supply from the *margins* of the band of vascular tissue. By this anatomical compulsion, and by the compact association of the three spikelets closely backing on the main axis, the abnormal position of the glumes is inevitably determined.

(*b*) *The Relation of Glume and Axis in the Abortive Spikelets.*

The abortive lateral spikelets of *Hordeum distichum*, L., var. *nigrum*, shown in Fig. 3, B, C, D, p. 515, are interesting as examples of extremely reduced shoots. The feature to which I want specially to draw attention is that the two or three bundles which enter the base of the shoot are all leaf traces for the first leaf (glume): the bundle for the continuation of the axis is given off at a later stage either from the median or from a lateral bundle of this leaf. And this shoot-bundle itself passes bodily into the second leaf, leaving the stem-apex non-vascular. Such a state of affairs is not easy to reconcile with conventional morphological ideas as to the relation of leaf and axis; to speak of the leaf (*glume* 1) in Fig. 3, B_2 and B_3, D_2 and D_3, as being 'given off' from the axis, scarcely represents the actual facts; the axis, rather, is given off from a minor bundle of the leaf.

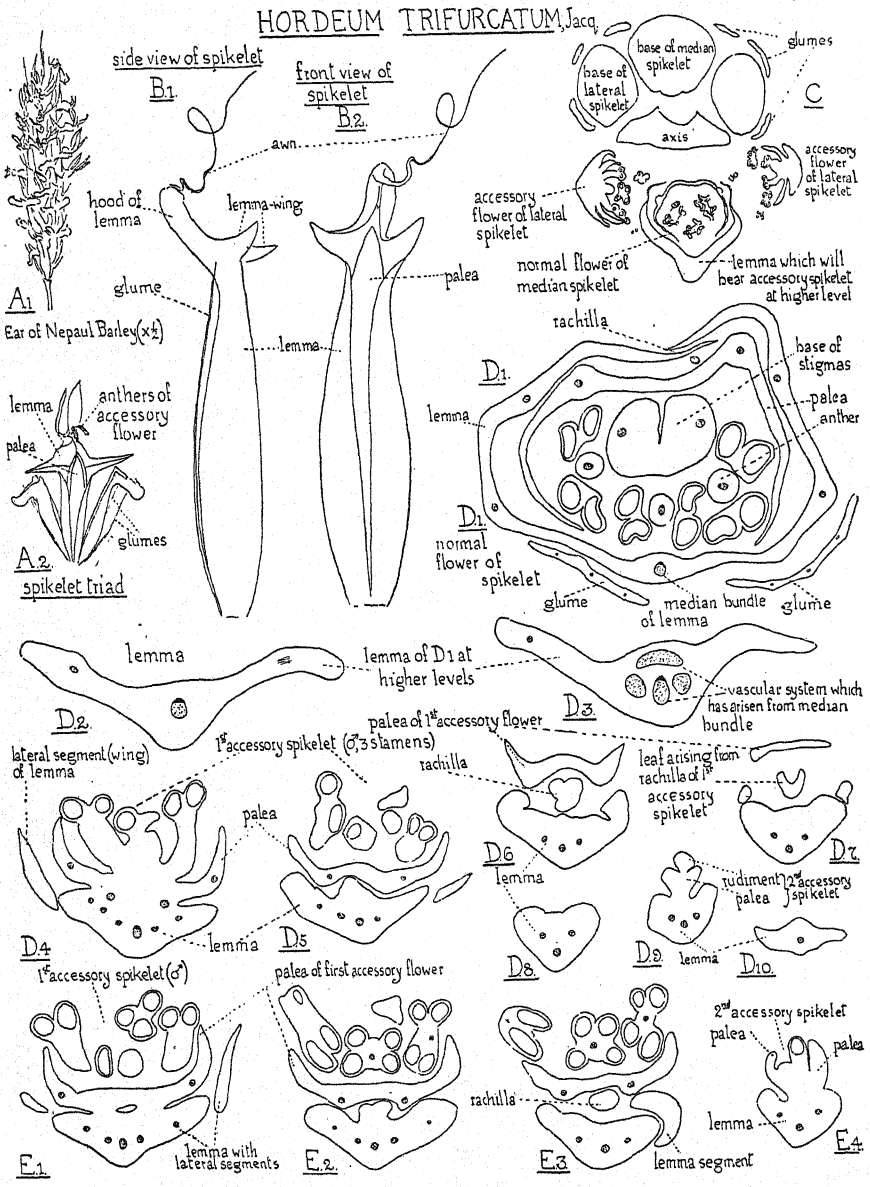


FIG. 5. *Hordeum trifurcatum*, Jacq., Nepaul Barley. A_1, inflorescence ($\times \frac{1}{2}$ *circa*) 2.7.2. A_2, triad of spikelets from such an ear as A_1, viewed from adaxial side, enlarged. Glumes of median spikelet invisible as they are abaxial. B_1, lateral, and B_2, adaxial, view of a spikelet, 6.7.27, much enlarged. C, transverse section (× 14) passing through two opposite spikelet triads. To the north the bases of three spikelets are seen below the level at which their lemmas become free, but above the level of detachment of the glumes. To the south the normal median flower is cut through its anthers; at a higher level an accessory flower will arise from the thickened median region of the lemma. As the lateral spikelets are shorter they are cut through their accessory flowers and the tops of the stamens of the normal flowers. (More than one section used and slightly reconstructed.)

4. The Vascular Supply of *Hordeum* Lodicules.

The two lodicules of *Hordeum* are like those of other Grasses in general appearance, but I have found the vascular system in most cases obscure, owing to the delicacy of the strands. However, in the normal flowers of *Hordeum trifurcatum*, Jacq., I have been able to trace the origin of the system, so I have figured it for this species in Fig. 4, D, p. 517. A single vascular strand is given off for each lodicule (D_1). These strands branch repeatedly (D_2 and D_3), so that in the base of the lodicule (D_4) the arrangement is scattered. Even at this level the bundles are becoming difficult to detect, and in this series they are indistinguishable from the ground tissue by the time that the lodicule becomes free. Fig. 4, C, shows the general arrangement of the strands in the lodicules of another spikelet, but they are procambial and difficult to see, so the details cannot be vouched for.

5. 'Nepaul Barley.'

(i) *Introduction.*

The curious heritable abnormality, 'Nepaul Barley' (Fig. 5, A, B, p. 520), has long been known under the names of *Hordeum trifurcatum*, Jacq., *H. cucullatum*, Koern., *H. Horsfordianum*, Wittm., &c., which may be regarded as sub-species of *H. vulgare*, L. A scattered literature has grown up round the subject, to which references will be found in the second edition of Penzig's 'Pflanzen-Teratologie' (16, vol. iii, pp. 472–3). Henslow's account of the inflorescence (13) and Royle's illustration of an ear as *H. Ægiceras* (18, pl. 97, Fig. 2) give some idea of its external morphology. But although attention has been so frequently drawn to the peculiarities of this form, the botanists who have dealt with it have mostly confined themselves to isolated notes on the external characters of such specimens as have fallen under their observation, and we are still very much in the dark as to the structure and meaning of this abnormality. Penzig, after summarizing the existing information, concludes that further work is needed, and it has seemed to me that serial sections of the abnormal inflorescences might perhaps help towards clearer ideas about these strange structures. So I have studied in this way a strain of Nepaul Barley grown at the Cambridge Botanic Garden from seeds supplied by Mr. F. L. Engledow, and I have made a few supplementary observations on other strains.

D_1–D_{10}, series of transverse sections from below upwards (× 48) through a lateral spikelet. D_1, normal flower at base of spikelet cut through anther, stigmas, and extreme tip of rachilla; lower down the development was entirely normal with lodicules and the usual four bundles in the gynaeceum. D_7 from four sections. For an account of the succeeding sections see text, p. 522. E_1–E_4, series of transverse sections (× 48) through the accessory spikelets of a lateral spikelet, 2.6.28. E_1 shows the detachment of the lemma wings; in E_2 a third lobe is detaching, and the lemma of its first accessory flower has become free. E_4, second accessory spikelet (rudimentary).

(ii) *Observations.*

The spikelets of Nepaul Barley are normal in their basal region and produce flowers there as usual. But the flowering glume or lemma behaves in an altogether exceptional way in its distal region. The mature lemma (Fig. 5, A_1, A_2, B_1, B_2, p. 520), instead of terminating simply in an awned apex, is trifurcated, and the middle lobe is hooded so as more or less to conceal the ventral surface, and is generally bent backwards; *within the hood are one or more accessory spikelets, produced as outgrowths from the lemma.* In order to try to understand this anomalous and complex structure I have cut serial sections of young inflorescences at a stage before hooding has become a conspicuous feature, because in these early stages the relations of the parts are less liable to be obscured than in older ears. Fig. 5, C, p. 520, shows the general appearance of a section through an inflorescence of Nepaul Barley; the triad to the south is cut through the normal flower of the median spikelet and through the accessory flowers of the lateral spikelets, which are shorter and thus carry their accessory flowers at a lower level. The history of a spikelet can best be followed in such a series as Fig. 5, D_1–D_{10}, which passes upwards from below through a lateral of a triad in which each lateral bore an accessory male spikelet, while the median spikelet bore an accessory hermaphrodite spikelet. In D_1 the normal basal flower of the lateral spikelet is shown. In itself and in its relation to glumes and lemma it diverges in no way from a typical *Hordeum* spikelet. Nor is there anything exceptional about the lemma in this region. In D_2 the lemma is cut at a higher level, and it will be seen that the midrib, which is somewhat thick, is traversed by a single collateral bundle. Higher still (D_3) this bundle branches and produces a vascular ring, destined to supply the accessory spikelet. In D_4 we have reached the accessory flower, which is male and contains three stamens. The lemma gives off a lobe on either side (D_4 and D_5). The two-bundled palea for the accessory flower bears the normal relation to its own flower, but is placed with its *back* to the lemma of the normal spikelet from which it arises. My sections are all so young that the palea bundles are unlignified, and hence the orientation cannot be determined, but in a hand-section from an older spikelet I have seen a palea strand with its xylem in the expected position on the side facing the flower. At a higher level the rachilla of the accessory spikelet is detached in the normal position with regard to the palea (D_6) and this rachilla terminates in a leafy structure (D_7) which we may interpret as the lemma for a non-existent flower. Higher still a second accessory spikelet is developed from the lemma (D_9) and in this the flower is again normally placed in relation to its own rudimentary palea. This series may be compared with the series E_1–E_4, in which essentially the same course of events occurs, with minor differences. It is striking that the single colla-

teral bundle of the lemma midrib should serve as the source of supply for all the remarkable developments of the lemma apex; I find this to be the case even in well-developed triads. Fig. 6, A_1, shows a section cut at the base of a spikelet in which the lemma before its detachment is supplied

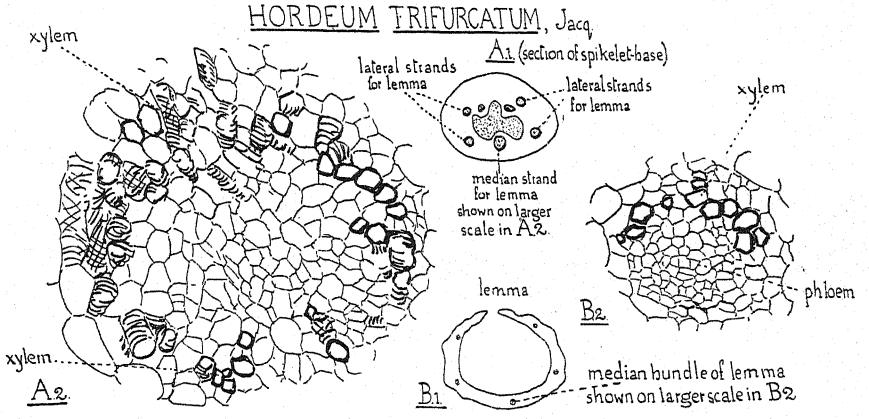


FIG. 6. *Hordeum trifurcatum*, Jacq. A_1, transverse section of the base of the median spikelet of a well-developed triad below detachment of lemma (× 14) to show the five concentric bundles destined for the lemma. A_2, the median bundle of the lemma in A_1 on a larger scale (× 327) to show concentric structure. B_1, transverse section close to the base of the free region of the lemma (× 14). B_2, collateral median bundle of the lemma of B_1, on a larger scale (× 327).

by five concentric bundles; the median bundle is drawn in greater detail in A_2. But when the lemma becomes free (B_2) the midrib is reduced to the normal collateral type (B_2).

The accessory flowers are not all unisexual like those drawn in Fig. 5, D and E, p. 520. So far as I have seen, it is characteristic that the median spikelet should produce a hermaphrodite accessory flower, while those of the lateral spikelets are male. Fig. 7, A_1, p. 524, shows a hermaphrodite flower with a central gynaeceum. Its rachilla produces another leaf before dying out. My sections are so young that it is impossible to be certain whether the essential organs would be fully functional, though they look quite normal; Wittmack (21) has recorded the occurrence of an accessory grain in two spikelets of a race of Nepaul Barley which he examined.

I have not detected lodicules in the accessory flowers in any of the sections of my Cambridge material; this may possibly be due to the plane in which they are cut, for I have observed two quite normal lodicules in sections of an accessory flower from a specimen grown at Kew.

In the young spikelets studied, the hooding of the lemma is not yet conspicuous feature, but it can sometimes be seen. In the series Fig. 7, E_1–E_8, for instance, the palea is clearly hooded at the apex (E_2), and the lemma slightly so (E_8). In one spikelet I found a lemma which bifurcated at the tip and produced two accessory flowers (more or less fused at their bases)

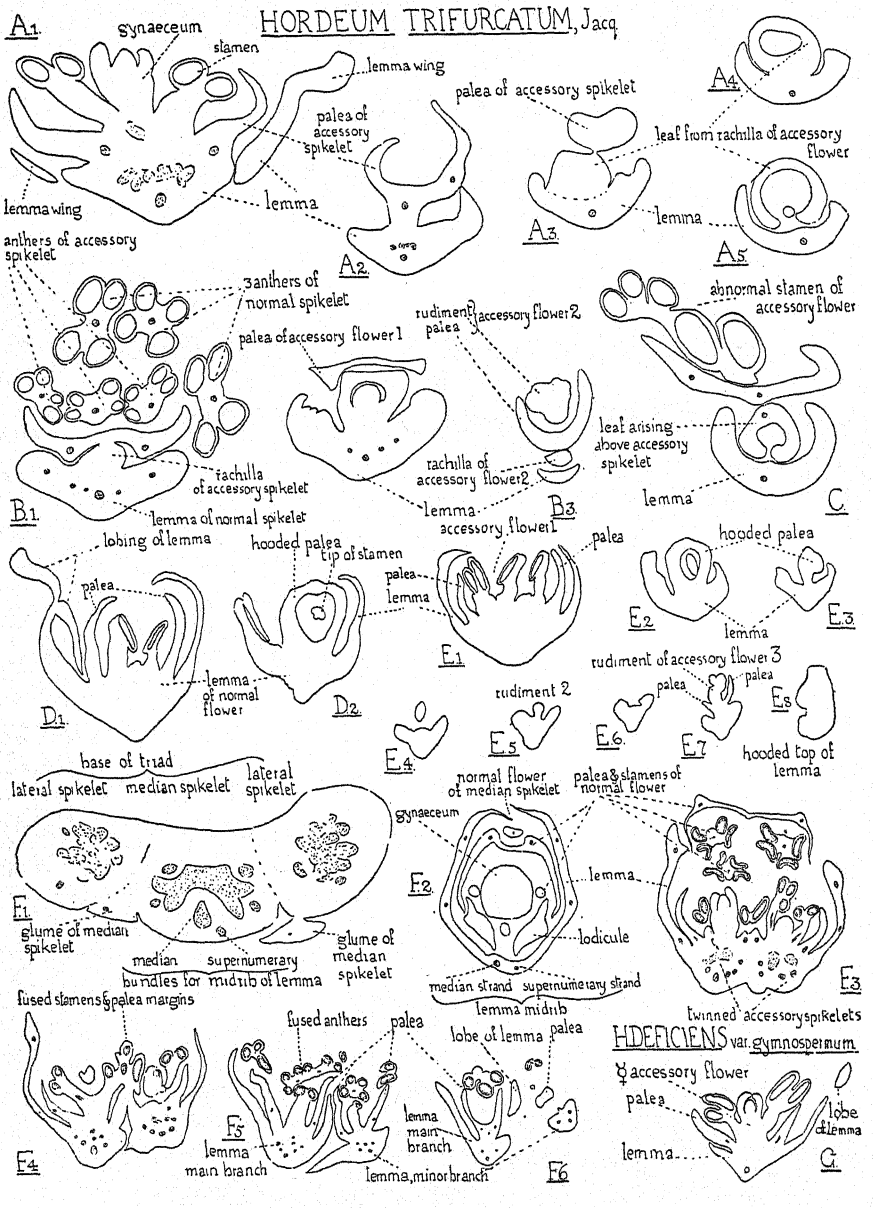


FIG. 7. *Hordeum trifurcatum*, Jacq. A_1–A_5, series of transverse sections (× 48) through the young accessory spikelet of the central spikelet of a triad, 2.6.27. · A_1 shows gynaeceum and wings of lemma. Rachilla of accessory spikelet gives off another leaf above the flower. B_1–B_3, transverse sections (× 48) from a series (2.6.27) through the accessory flowers of a lateral spikelet. B_1, anthers of normal and accessory flowers. B_3, palea and rudiment of second accessory flower. Rachilla is free for a minute distance above accessory flower 2. Owing to loss of sections between B_2 and B_3 the parts of the flower rudiment B_2 cannot be identified with certainty. C, transverse section (× 48) of the accessory flower of a median spikelet to show a stamen with an extra pollen sac. This flower has in addition two other stamens and a gynaeceum. Above the accessory flower the rachilla is

instead of one (Fig. 7, F_1-F_6, p. 524). I was interested to find that these twinned flowers arose from a lemma which had in its midrib an extra bundle which could be traced down to the very base of the spikelet below the detachment of the lemma (F_1). That such abnormalities depend on the fact of an extra vascular supply, for some unknown cause, entering the organ, is rendered probable, I think, by a series of sections illustrated in an earlier figure (Fig. 4, E_1–E_4, p. 517). In the normal spikelet of *H. trifurcatum*, the one-bundled rachilla terminates a little distance above the flower, without further developments. But in the spikelet figured, and in one other, I found that the rachilla became hollowed out into a leaf-like structure, and in each of the two this continued development was associated with an extra vascular supply. It will be seen that the rachilla, instead of receiving one bundle, is supplied from the very base (E_1) with three bundles. When it becomes free (E_2), it contrasts markedly with the normal rachilla, which is smaller and supplied with one bundle only (E_3).

For comparison with Mr. Engledow's strain of *H. trifurcatum* I have examined another strain supplied by Mr. R. S. Pearl, and I find that this has the same general structure. I have also cut sections of another race of Nepaul Barley grown at the Cambridge Botanic Garden from seeds received from Mr. Engledow—*H. deficiens*, Steud., var. *gymnospermum*. Here again I find that the accessory flowers, Fig. 7, G, p. 524, which in this strain are confined to the median spikelets, are closely similar to those of *H. trifurcatum*. But there is probably a good deal of variety among the strains classed as Nepaul Barley, and further study might very likely reveal other types of structure; my observations in no way pretend to cover the whole ground.

In conclusion I may say that, in the strains of *H. trifurcatum* which I have examined, the principal feature in the structure of the accessory spikelet is that it invariably has a two-bundled palea placed with its back to the ventral surface of the lemma (flowering glume) of the normal spikelet from which it arises; the rachilla of the accessory spikelet occurs between the palea of the accessory flower and the lemma of the normal flower. I have never observed the development of a second flower from this rachilla, but it

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hollowed into a leaf-like structure facing the lemma. Above the level of this section both the lemma and the leaf facing it die out without further developments. D_1 and D_2, transverse sections through an accessory spikelet (× 24) to show hooding of palea and lobing of side of lemma. Higher up, hood of palea closes completely. E_1–E_8, series of transverse sections through the tip of a lemma bearing accessory flowers, 20.6.27 (× 24). E_1, from more than one section. E_2, through hooded tip of palea. The papilla in E_5 is a spikelet rudiment which does not free itself; it is succeeded in E_7 by a better developed rudiment. E_8, hooded apex of lemma. F_1–F_6, series of transverse sections showing the origin of two hermaphrodite accessory spikelets from the apex of one lemma belonging to the median spikelet of a triad (× 24). F_1, through the base of a triad to show, in the median region of the median lemma-base, the supernumerary bundle which is responsible for the extra spikelet. F_2 passes through the normal flower of the median spikelet, and shows the two midrib bundles of the lemma. In F_4 there is some fusion between the twin spikelets, while in F_5 they are becoming free. G, *Hordeum deficiens*, Steud., var. *gymnospermum*. Transverse section of an accessory hermaphrodite flower borne by the lemma of a median spikelet (× 24).

sometimes gives rise to a leaf structure which might represent the lemma for a second non-existent flower, e. g. Fig. 5, D_7, p. 520. A second rudimentary accessory spikelet may be produced above the first from the lemma of the normal spikelet. The accessory flowers never have lemmas of their own.

(iii) *Discussion.*

There does not appear to have been much discussion about the meaning of the abnormal structure of the spikelet in Nepaul Barley. Penzig (16) thinks that the question of its morphology must be left open until further information is available. Worsdell (22) points out that the view generally adopted has been that the accessory spikelet arises adventitiously from the upper surface of the lemma (flowering glume), but he also suggests the possibility that the anomaly may consist merely in the fusion of an axillary product with its subtending bract. The latter alternative seems improbable. It would involve the curious phenomenon of buds of two orders—a flower and a spikelet—arising in the axil of the same leaf member. Leaves axillant to spikelets are rare in the Grasses, but in the unusual cases drawn in Fig. 1, A–C, p. 508 (cf. also Fig. 4, A_2, p. 517), we see the relative position of a spikelet triad and its axillant leaf. Here the palea of the central flower lies on the side remote from the axillant leaf, but in Nepaul Barley the accessory palea and rachilla are interposed *between* the flower and the lemma from which it arises (cf. Fig. 5, D_1–D_{10}, p. 520). On general morphological grounds it is therefore difficult to regard the lemma as the axillant leaf of the accessory spikelet. Moreover, the midrib of the lemma with its single collateral bundle (Fig. 5, D_2, p. 520, Fig. 6, B_1 and B_2, p. 523) shows no anatomical sign that it is the result of the fusion of two organs.

It should be remarked in passing that it may perhaps seem surprising that a single collateral bundle (the median bundle of the lemma) should be the only supply channel for the accessory spikelet or spikelets. But we may find a parallel for this anatomical economy in the spikelets of other Grasses. The sterile spikelet of *Cynosurus cristatus*, L., is served by a single collateral bundle which is responsible for a number of distichous leaf traces (5, pp. 181–2), while in *Luziola Spruceana*, Benth., there is a single collateral bundle in the spikelet axis below the flowering glume in both the male and female spikelets (6, pp. 396, 398). I have also seen the same thing in the base of the spikelet of *Alopecurus pratensis*, L. Here the two glumes, the lemma, the stamens, and the gynaeceum are all supplied by a branch system arising from this one bundle.

If we reject the view that the accessory spikelet in *H. trifurcatum* is a shoot axillary to the lemma, we must, I think, accept the fact that no neat morphological interpretation can be provided for it. We can only say that the lemma behaves to the accessory spikelet exactly as if it were that

spikelet's parent axis. The absence of an axillant leaf is not surprising, as the spikelets of Grasses are hardly ever subtended by such a leaf. But these reduced accessory spikelets of *H. trifurcatum* are peculiar in that they possess neither outer glumes nor lemma. Their well-defined and regular adventitious origin from the ventral surface of the lemma midrib makes one realise the artificiality of the hard-and-fast morphological distinction between the axis and the leaf.

We may perhaps look upon the production of accessory spikelets from the upper region of the lemma as another symptom of that capacity for apical overgrowth expressed also in the awn-production at the tips of glumes and lemmas which is responsible for the 'beard' of the Barley ear.

6. *PARIANA.*

The South American genus *Pariana* has certain features in its inflorescence which recall *Hordeum*, and its 'glumes'—as in *Hordeum*—have been claimed by Schenck (19) as being in reality sterile spikelets, and by Schuster (20) as representing halves of the first glume, the second glume being abortive. Sections and external views of the curious inflorescences have been figured by Goebel (10), Pilger (17), and Schuster (20), but their diagrams do not give all the information necessary to elucidate the structure and origin of the glumes, so I have re-examined such species as I could obtain.

An incomplete inflorescence of *P. campestris*, Aubl., is shown in Fig. 8, B_1, p. 529. Each partial inflorescence (Fig. 8, B_2) consists of an apparent whorl of five male spikelets whose bases are flattened and fused to form a cup-like involucre which encloses the solitary female spikelet. The rim of the cup expands and divides into the five male spikelets, each accompanied laterally by two glumes which are dotted in Fig. 8, B_2. All the species of *Pariana* which I have attempted to microtome have proved highly resistant to sectioning. Only material collected for herbarium purposes has been available, and it has not happened to include any young inflorescences. The best results I obtained were with two dried spikelets of *P. campestris*, more than eighty years old, which I treated for twenty-two days with dilute potash before infiltrating with paraffin. One of them gave a fairly complete series, and the conclusions from it were in general corroborated by the second less complete series. Fig. 8, A_1–A_4, shows the successive detachment of the involucral segments: these are numbered in the order at which at higher levels the bases of the male flowers come into view. In A_4 the segments of the involucre and the base of the female spikelet are all detached from the axis. The female spikelet becomes free from the axis after segments 3, 4, and 5, but before segments 1 and 2. In *Pariana lunata*, Nees, I have also observed that it becomes free before the two segments facing it on the opposite side of the axis. In A_5

the structure of the female spikelet can be seen. It has, as Goebel and Schuster have already shown, two outer glumes, a lemma and palea, three lodicules, and six structures which appear to be stamen rudiments. Fig. A_6, which is cut at a higher level, passes through the stigmas of the female flower and through the stamens of the five male flowers, while A_7, which is cut still higher, only shows the apex of the female spikelet beside the axis, which is apparently enlarging in preparation for the development of the next partial inflorescence.

The details of the origin and development of the male spikelet can be followed more easily in Fig. 9, A_1–A_4, and B, p. 530. In A_1 the glumes form wings at the sides of the spikelet base, and in A_2 they have separated and the lemma forms the basal cushion so common in Grasses (4). In A_3 the palea, the two front lodicules, and the stamens are coming into view. In A_4 the parts are all complete and separate, and it is seen that the male flower, like the female, has three lodicules. The preservation is not good enough to allow the number of stamens to be counted, but it appears to exceed twelve.

The vascular anatomy of the glumes is difficult to follow in my material of this species, but it will be seen from Fig. 8, A_5, p. 529, in which the bundles are roughly indicated, that the segments of the involucre (the bases of the male spikelets) have a highly dorsiventral structure in correlation with their flattened shape. The strands form one series except in the median region of the segment, where there is an approach to radial symmetry A similar type of structure is seen in the flattened bases of the spikelets of *P. zingiberina*, Doell (Fig. 9, E, p. 530). But the dorsiventrality is sometimes even more pronounced. In *P. lunata*, Nees (Fig. 9, D), and *P. imberbis*, Nees (C_1–C_5), the involucral segments may have a single series of strands only. The detachment of a glume from such a segment can be followed in C_1–C_5. It arises marginally like the glume of *Hordeum*.

There seems to be no reason whatever to interpret these paired glumes either as sterile spikelets or as halves of a single glume. They show no characters that are axial rather than foliar; and they are so remote from one another in origin that it is not reasonable to suppose that they are members of one organ. Their anomalous position to right and left of the spikelet (instead of to front and back) can be accounted for much more simply on the same lines as in *Hordeum* (pp. 512, 513). The flattened form and the dorsiventral anatomy of the base of the male spikelet necessitates a lateral origin for the first appendages that are detached. So I am led to conclude that the glumes of *Pariana*, like those of *Hordeum*, are the first and second empty glumes, equivalent to those of other Grasses.

Doell (9) long ago suggested that the partial inflorescence of *Pariana* might be compared with two opposite spikelet-triads of *Hordeum*—the female flower and the male to either side of it representing one triad, and

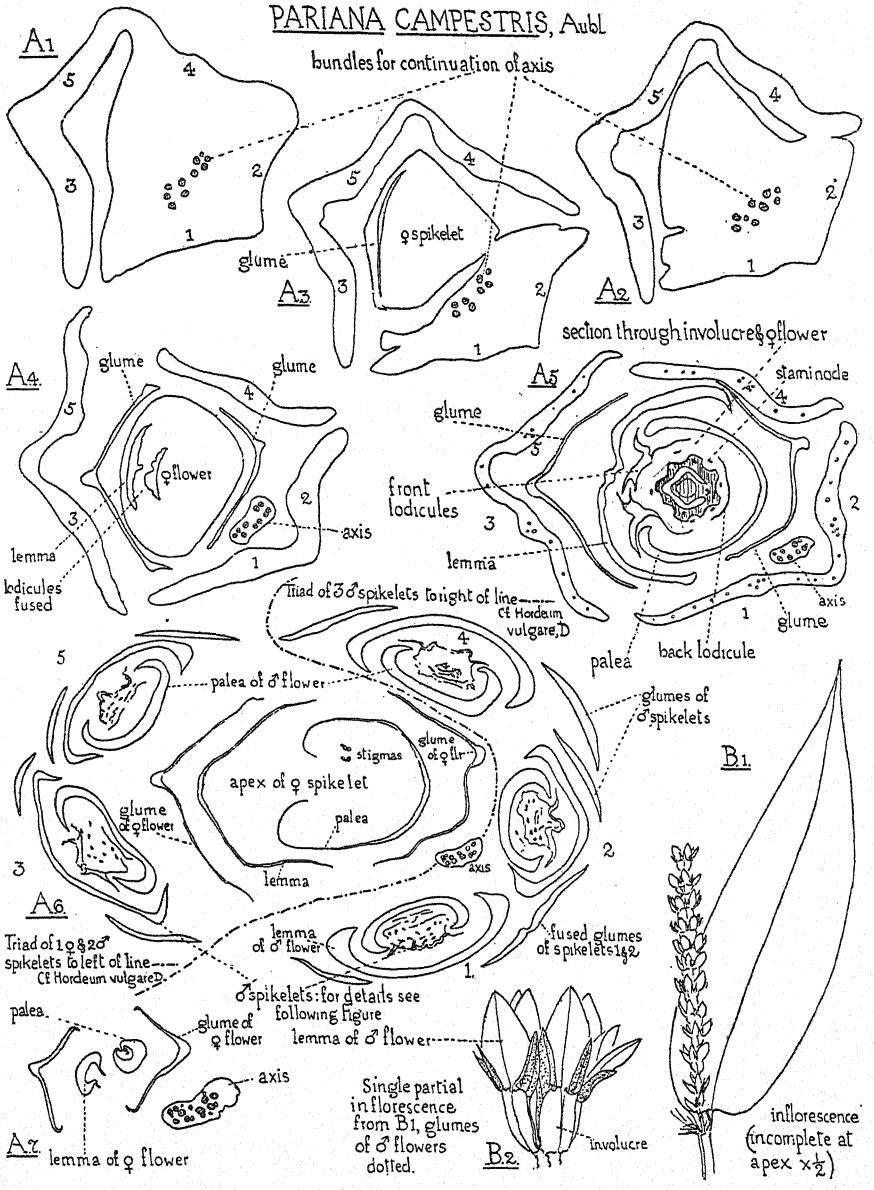


FIG. 8. *Pariana campestris*, Aubl. A, from Surinam, 1841, Hostmann, 20. A_1–A_7, series of transverse sections from below upwards through a partial inflorescence (× 14). Except in A_5, all bundles omitted except those destined for the continuation of the axis. The male spikelets are numbered in the order in which the bases of the flowers come into view. In A_6, which shows the four male spikelets surrounding the tip of the female spikelet, a broken line indicates the boundary between the two hypothetical spikelet triads; this diagram should be compared with *Hordeum vulgare*, L., Fig. 1, D, p. 508. B_1, inflorescence, incomplete at the apex, from Surinam, Camb. Bot. School Herbarium (×½ *circa*). B_2, single partial inflorescence from B_1, with glumes dotted (enlarged).

the three male spikelets on the opposite side of the axis representing the other triad. This view appears to me well founded, and in Fig. 8, A_6, p. 529,

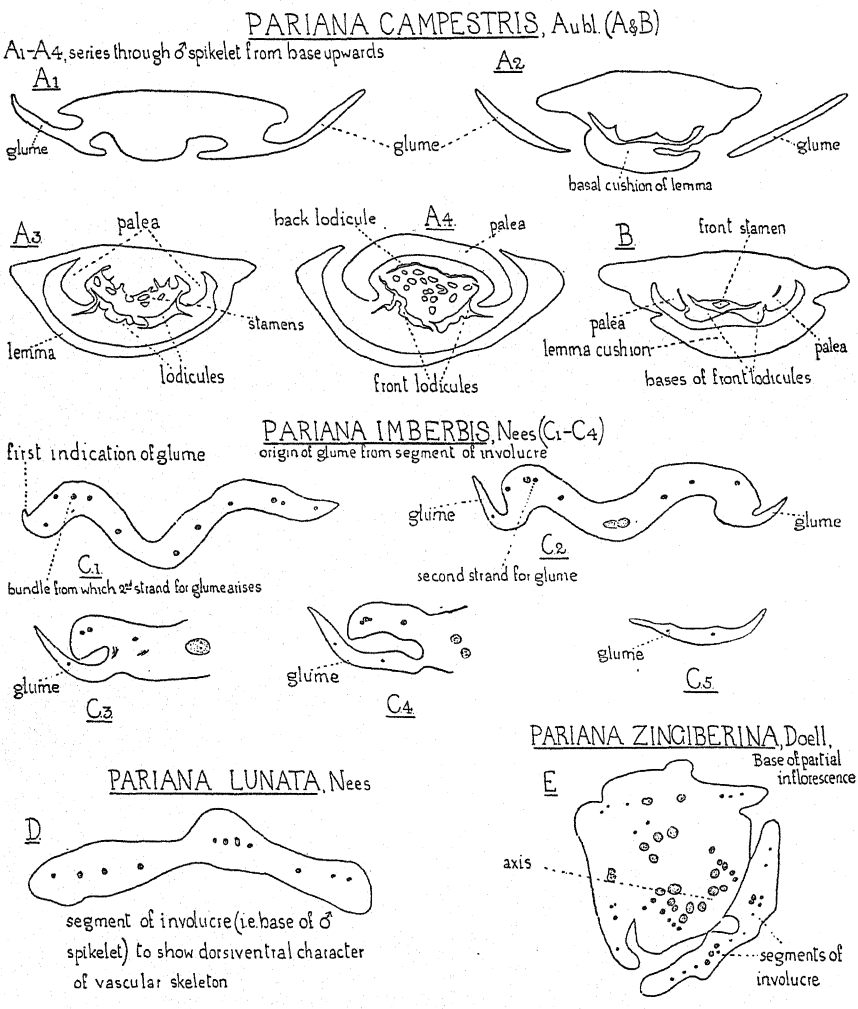


FIG. 9. A and B, *Pariana campestris*, Aubl., Surinam, 1841, Hostmann, 20. A_1–A_4, series of transverse sections between Fig. 8, A_5 and A_6, p. 529, showing structure of *male spikelet* 1 in greater detail (× 24). B, *male spikelet* 2 at level between A_2 and A_3 (× 24). C_1–C_5, *Pariana imberbis*, Nees, prope Barra, Prov. Rio Negro, R. Spruce, 1855; series of transverse sections from below upwards (× 24) to show origin of glume from involucral segment. Bundles difficult to follow and may not be represented exactly. Before the origin of the second glume bundle it appears that the bundle from which it is derived fuses with the adjacent lateral. D, *Pariana lunata*, Nees, French Guiana. transverse section of an involucral segment (× 24). E, *Pariana zingiberina*, Doell, Altson, British Guiana, No. 314, transverse section of base of a partial inflorescence (× 14).

I have indicated the boundary between the presumed triads by means of a broken line. This diagram should be compared with the section of

H. vulgare (Fig. 1, D, p. 508) in which I have also inserted a broken line in the corresponding plane.

7. Summary.

The points in the morphology of *Hordeum* and *Pariana* dealt with in the present paper may be summarized as follows:

1. The ear of cultivated Barley (*H. vulgare*, L.) is shown to bear at its base a reduced foliar member which may be called the collar leaf. The collar leaf bears a shoot in its axil—a spikelet triad—and this shoot may produce a prophyll, an organ which I have not found elsewhere in the inflorescences of *Hordeum*. The collar leaf is peculiar in being entirely non-vascular, though it is relatively massive. It is the most highly developed example of a non-vascular leaf which I have met with among the flowering plants (pp. 509–511 and Fig. 1, A, B, C, p. 508).

2. From a comparative study of the partial inflorescences of *Hordeum* and *Pariana*, it is concluded that the lateral 'glumes' are neither sterile spikelets nor halves of the first outer glume, but are true glumes, corresponding to the first and second outer empty glumes of other Grasses. It is claimed that their peculiar lateral position is brought about by the excessively dorsiventral character of the axis or its branches, which results in the vascular supply of the triad of *Hordeum* and of the male spikelet of *Pariana* diverging from radial symmetry, and forming an arc or band. The first organs to be developed from the spikelet base arise from it at the level where it is still thoroughly dorsiventral and their vascular system is naturally derived from the margins of the arc. Their unusual right-and-left position is thus mechanically inevitable (pp. 511–519, Figs. 2, A and B_1, p. 510, and Figs. 9, C_1–C_4, p. 530).

3. The anatomical examination of *Hordeum* spikelets has incidentally brought to light some evidence for the view that an unlignified bundle may be an efficient conducting channel (p. 513).

4. In *H. trifurcatum*, Jacq., the only species in which the history of the vascular supply for the lodicules has been followed, it has been found that each of these organs receives a single vascular strand. Before the lodicule becomes free, this strand gives rise by repeated branching to a number of small, delicate, scattered bundles (pp. 521 and Figs. 4, C and D, p. 517).

5. Doell's view (9), according to which the partial inflorescence of *Pariana* is comparable with two triads of *Hordeum*, is confirmed; the relation of the two can be understood by the comparison of Fig. 1, D, p. 508, and Fig. 8, A_6, p. 529.

6. It is shown that in the abortive lateral spikelets of *H. distichum*, L., var. *nigrum*, the relation of leaves and axis does not accommodate itself

satisfactorily to current morphological ideas (p. 519 and Figs. 3, B, C, D, p. 515).

7. As a result of the study of serial sections of the inflorescence of Nepaul Barley, *H. trifurcatum*, Jacq. (pp. 521–527 and Fig. 5, p. 520, Fig. 6, p. 523, Fig. 7, p. 524), it is shown that the accessory spikelet developed from the lemma (flowering glume) of the normal spikelet is apparently not an axillary product fused with this lemma. It arises as an adventitious outgrowth from the ventral face of the lemma midrib, which behaves—in relation to the accessory spikelet—as though it were its parent axis. The accessory spikelets maintain a highly definite and regular relation to the lemma which bears them. The rigid distinction drawn in formal morphology between leaf and axis can only be maintained by shutting one's eyes to such inconvenient exceptions.

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Mycorrhiza as a Possible Determining Factor in the Distribution of the Strawberry.[1]

BY

PHILIP R. WHITE.

With six Figures in the Text.

INTRODUCTION.

ALL of the strawberries in general cultivation to-day seem to be descended originally from crosses between the Chilean strawberry (*Fragaria Chiloensis*) and the common strawberry of the eastern United States (*F. Virginiana*). *F. Virginiana* has quite a wide range and shows no striking peculiarities in distribution. *F. Chiloensis* on the other hand has an extraordinarily sharply defined and restricted distribution, and appears to be very exacting in its environmental requirements. Attempts to introduce it into the eastern United States have consistently failed. The plants thrive the first year, but come out of the winter greatly weakened, and usually die off completely at the end of the second year. As one of the probable parents of most of our commercial varieties of strawberries, it is highly desirable to be able to cultivate this species in various localities, and these apparently obligatory restrictions on its distribution have long been a puzzle to horticulturists.

FUNGI IN THE STRAWBERRY.

In the preparation of material for a study of the vascular anatomy of the strawberry (*Fragaria* spp.), the roots of many of the plants were found to harbour fungi of one or possibly more species. These fungi bear typical arbusculate haustoria similar to those of the endotrophic mycorrhiza of other plants (Rayner, 18–19, Gallaud, 5). Such fungi were first noticed in roots of the varieties Howard 17 and Progressive. Jones (10) reports

[1] The work here reported was done in 1925–1926 while the author was employed as Microscopic Technician, Office of Horticultural Investigations, Bureau of Plant Industry, U.S. Department of Agriculture. It was incidental to an anatomical problem (White, 23), and at that time was not considered of enough consequence to warrant its publication. In view of recent work indicating its importance (O'Brien and M'Naughton, 15, Plakidas, 16, and others), it now seems desirable to give these results to the scientific world.

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a fungus in the roots of *F. Virginiana*, which is probably similar. Further investigation disclosed the same type of fungus in *F. Nilgherrensis* and *F. platypetala* and in the horticultural varieties Rockhill, Dunlap, and Ettersburg 121, and in especial abundance in *F. Chiloensis*.

The finding in *F. Chiloensis* and in other species of strawberries of a fungus having many of the characteristics usually associated with a symbiotic relation suggests the possibility that the survival and transmission of this fungus in its original relationship to the host may prove to be a determining factor in the distribution of that host. There are two possible changes which may enter into this relationship upon transplantation. Either (1) the fungus itself may be destroyed annually by the host (Frank, 4, Magrou, 13) or be otherwise unadapted to its new soil and climatic conditions, or (2) the fungus, instead of being beneficial or neutral, may become pathogenic (Knudson, 11, Bernard, 1). When this problem arose, therefore, it was suggested that a careful investigation should be made as to the character of the fungous and autotrophic plant associates of the Chilean strawberry, and of the sort of soil in which it is found. It was suggested that probably it would be found to inhabit acid soils in company with the Ericaceous plants which are particularly common in a part of its range (N. Pacific seaboard). This seemed particularly probable in the light of the work of Knudson (11) demonstrating the necessity for a certain pH value for the germination and development of Orchid seeds and seedlings, which value is maintained in nature largely through the activity of the associated fungi, and in view of the similar high hydrogen-ion concentration required by the mycorrhizal Ericaceae (Coville, 3).

This latter suggestion was verified as concerns the coasts of Oregon and Washington during the summer of 1926 (G. M. Darrow). In Washington, Oregon, and Northern California *F. Chiloensis* occurs as a strictly beach plant. On the sand dunes it is often the farthest outpost of vegetation, growing on white sand, often forming pure stands. It rarely passes beyond the crests of the dunes, and was not observed higher than 60 to 70 feet above sea-level. Where it is found on the landward side of low dunes it is usually associated with plants characteristic of acid soils such as *Arctostaphylos uva-ursi*, *Gaultheria shallon*, and *Vaccinium ovatum*, which are known to be mycorrhizal plants. It has seldom been found in association with non-acid-soil plants, being either in pure stands, in Fragaria-Ericaceae associations, or occasionally in Fragaria-Grass associations. Georgeson (6) reports *F. Chiloensis* from Alaska 'along the beaches for hundreds of miles as far west as the Kenai Peninsula, growing with blueberries'.

In contrast to this strictly beach habit *F. Chiloensis* is reported in South America in 'desert and meadow formations, gravel and rubble of river banks, &c.' as far as 46° S., and 'not seldom . . . on Valle Pica' (16,000 ft.), 'Valle Friar, Valle Koslowsky' (all volcanoes), and in Andean

Patagonia at Puerto Blest (3,000–4,000 ft.), as well as along the beaches (Skottsberg, 21). Apparently referring to this species Prince (17) states that he has picked it on the Mexican Cordilleras and 'bordering the roads of lava . . . (in) the vast volcanic mountains of Jalapa'. The U. S. National Museum has specimens from Allantaytambo (10,000 ft.) in Peru, from 'Selkirk's Lookout' in Chile, and from an uncertain locality in Bolivia. It also has specimens from another distant region, the Mauna Kea (4,500 ft.), the Haleakala crater (6,000–10,000 ft.), and the Hualalai (7,000 ft.) in the Hawaiian Islands. Mann (14), Hillebrand (9), Henshaw (8), Guppy (7), and Campbell (2) also report it from Hawaii, the last three in connexion with its introduction there from the American continent by the Pacific Golden Plover (*Charadrius dominicus fulvus* Gmelin). Kordzumi (12) reports under the name *F. Hayatae*, but corresponding to the description of *F. Chiloensis*, a strawberry from Formosa found on Mounts Gonzan, Morrizan, Tozan, and Ekitaizan.

The Chilean strawberry is, then, restricted, as far as information is available, to three types of associations: (1) pure stands on clean, spray-washed sand; (2) Fragaria-Ericaceae associations on acid soils; and (3) associations on volcanic soils. It seems surprising that this species has not been found on the volcanoes of Guatemala and Yucatan, but these have been none too thoroughly botanized. Such a clearly defined distribution is certainly very suggestive of a physiological relation worth examining closely, and the occurrence of mycorrhiza gives a suggestion of one direction in which to look.

The Fungus in the Host.

The root system of the strawberry is made up of (1) large fleshy adventive roots, and (2) slender, fibrous branch roots (see White, 23), which differ in function. Fungi occur in both types. The hyphae enter under the root-cap of the larger type before differentiation into cortex and central cylinder has begun, and apparently remain almost dormant in the central cylinder until this is completely developed. Here they are from 10 to 15 μ in diameter and very sparingly branched. They remain in the central cylinder until the formation of branch roots when they again become active. They pass into the branch-root primordia and thence into the cortex of the newly formed rootlets, where they keep pace with the growth of the latter, occupying a narrow region just outside the endodermis (Fig. 1). In these branch roots the character of the fungus changes completely, it becomes highly branched and sends out numerous haustoria which penetrate and coil within the cells, often immediately around the host nucleus (Figs. 2–4). The hyphae vary greatly in outline, but are in the main from 5 to 7 μ in diameter. The nuclei are clearly distinguishable and quite evenly distributed along the hyphae, but within the host it was impossible

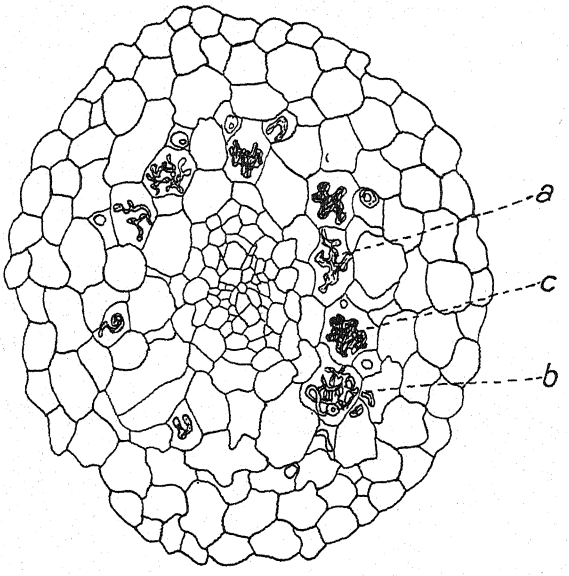


FIG. 1. Transverse section of young fibrous root of strawberry (Howard 17), showing distribution of mycelium: (*a*) early coiling of large hyphae; (*b*) hyphae finely divided; (*c*) final amorphous degeneration of the mycelium. × 200.

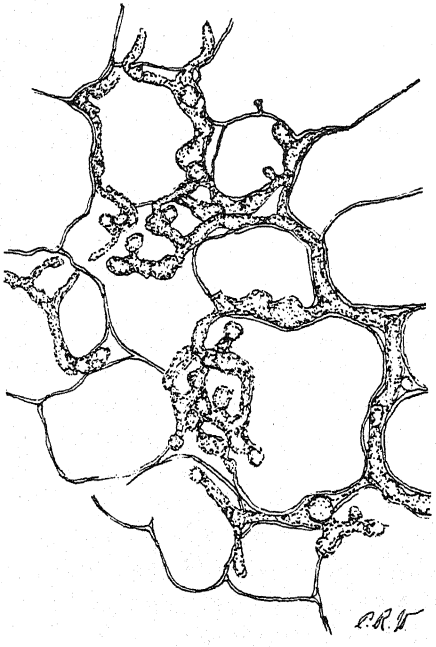


FIG. 2. Hyphae of fungus in cells of the root cortex of *Fragaria Chiloensis*, showing intercellular and intracellular position and knob-like haustoria. × 500.

to identify cross walls with certainty (see description of fungus in culture). The haustoria are often very irregular in outline and covered with knob-like processes (Fig. 2). By continued ramification these haustoria give rise to

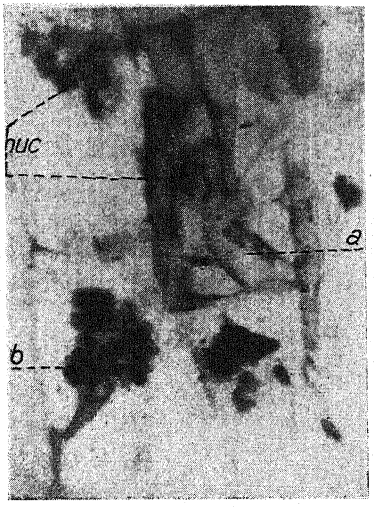


FIG 3.



FIG. 4.

FIG. 3. Microphotograph (var. Progressive), showing at 'a' the large hyphae coiled around the host nucleus (*nuc.*), and at 'b' a typical arbuscule after digestion of the finer ramifications. × 670.

FIG. 4. Microphotograph (var. Progressive). (*a*) hyphae coiled around the nucleus of the host cell. (*b*) finely divided to form a typical arbuscule. At (*c*) the host nucleus has begun the process of digestion of the fungus. Its exact structure is indistinguishable. × 670.

the 'arbusculae' so characteristic of endotrophic mycorrhiza (Fig. 3–4). These arbusculae finally degenerate (or are digested?) leaving an amorphous mass attached to the cell-wall by a stalk (Fig. 3).

There is no conclusive evidence that the root-hairs serve as points of entrance for the fungus, although fine hyphae sometimes occur within them. The mode of branching of such hyphae is such as to indicate that growth was taking place toward the tip of the root-hair and not away from it, suggesting that they must have come from within the root proper. Their identity with the arbusculate mycorrhizal fungus is yet to be definitely established, but the evidence seems to point to these slender hyphae as being branches sent out from an already firmly established stock, and more nearly comparable to the fine branches sent into the leaves and floral organs by the mycorrhizal fungus of the Ericaceae (Rayner, 19).

Although the three phases described above may represent three different organisms, it seems more probable that they are three phases in the life cycle of the same organism: (1) the large, semi-passive, infecting

mycelium; (2) the smaller, more complex, haustorial phase attacking the host; and (3) the very fine hyphae in the assimilating organs of the host.

Isolation of the Fungus.

Attempts have been made to isolate the fungus in question on artificial media. Small fragments of cortical tissue were removed from the roots, the usual precautions being taken to prevent contamination from outside. These fragments were introduced into slant tubes of agar. Agar prepared with a boiled extract of strawberry roots, subsequently strained but not filtered, proved to be the best medium. In spite of all precautions many of the cultures gave growths of characteristic epiphytic fungi, which throws some doubt on the adequacy of the method. In most cases, however, where cultures were obtained, the fungus was of the *Phoma* type which, both from its manner of growth and the known symbiotic relation of

Table I.

Record of Isolations of Fungi from Strawberry Roots.

(Isolations marked with an asterisk (*) were of pycnidial strains.[1])

Culture No.	Host.		Fungus Isolated.	
8	*Fragaria Chiloensis*		*Phoma, sp.*	
9	,, ,,		,,	+ *Fusarium*
22	,, *virginiana*		,,	
28	Howard 17	*	,,	+ *Fusarium*
30	,, 17		,,	+ *Chaetomium*
32	Ettersburg 121		,,	+ *Fusarium*
36	,, 121	*	,,	
56	Howard 17		,,	
57	,, 17	*	,,	+ *Botrytis* (?), *sp.*
62	Ettersburg 121		,,	+ (unidentified)
89	*Fragaria Chiloensis*		,,	+ *Mucor*
93	,, ,,		,,	+ ,,
95	,, ,,		,,	+ ,,
96	,, ,,		,,	+ ,,
101	,, *virginiana*		,,	+ *Fusarium*
137	,, *platypetala*		,,	+ *Chaetomium*
141	,, ,,	*	,,	
181	,, ,,		,,	
182	,, ,,		,,	
186	,, *Nilgherrensis*		,,	
188	Rockhill		,,	
190	,,		,,	
194	Dunlap		,,	

[1] The writer is indebted to W. W. Diehl, of the Plant Disease Survey, U.S. Department of Agriculture for the identification of certain of these fungi. In his communication he states: 'It may be of interest to you that just five years ago at this time I isolated a *Phoma* (similar to or probably the same as no. 141) from strawberry roots at St. Paul, Minnesota.' It is pertinent to state that the writer's no. 141 was taken from wild strawberries (*Fragaria platypetala*) collected near the mouth of the Columbia River in Oregon.

other (?) species of *Phoma* in members of the Ericaceae, make it probable that this is the mycorrhizal fungus in question.

Table I gives in detail the results of the few successful isolations. Out of 141 cultures only 39 showed fungous growths of any sort. Of these

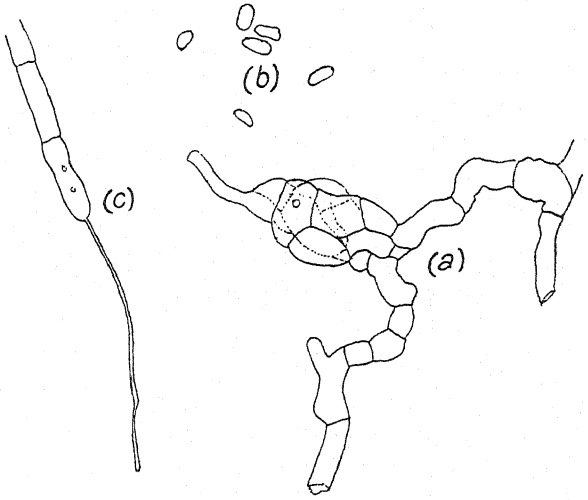


FIG. 5. (*a*) An early stage in the formation of a pycnidium of *Phoma* sp. from Ettersburg 121. The mycelium is typical of the older hyphae, showing the crooked, irregular outline, (*b*) pycnospores, (*c*) a large, septate hypha giving rise to a very attenuate, non-septate hypha. × 750.

4 were pycnidial strains of *Phoma*, 19 contained fungi with the vegetative characters of the known strains of *Phoma*, but had not formed pycnidia when last examined, and were in most cases contaminated with other fungi, 11 cultures showed growths of *Mucor* sp., 3 of *Chaetomium* sp., 1 of *Penicillium* sp., 1 of *Rhizopus* sp., and 1 of *Botrytis* (?) sp. Seven tubes contained fungi of doubtful identity. It will be seen that somewhat more than 50 per cent. of the cultures contained strains of *Phoma*, the remainder being mixed epiphytes. Control cultures made from yarrow and from an unidentified Crucifer, taken from the same plots as the strawberries, gave negative results.

The manner of growth of this *Phoma* on artificial media is peculiar, which perhaps accounts for the number of failures in culture. The hyphae spread out from the tissue fragment in all directions, closely pressed against the substratum but not penetrating deeply into it, and initially the mycelium is never aerial. Hence, in most cases where bacterial contamination accompanied inoculation the fungus was immediately prevented from gaining access to the substratum. Moreover, where there was also contamination by erect fungi such as *Mucor* and *Fusarium* the presence of the *Phoma* was entirely masked and could only be detected by microscopic examination.

The mycelium spreads out fan-like, and is so highly branched that it imparts a dull surface to the substratum without individual strands being detectable, except just at the margin of the infected area. The hyphae are hyaline or somewhat yellowish, but do not noticeably alter the colour of

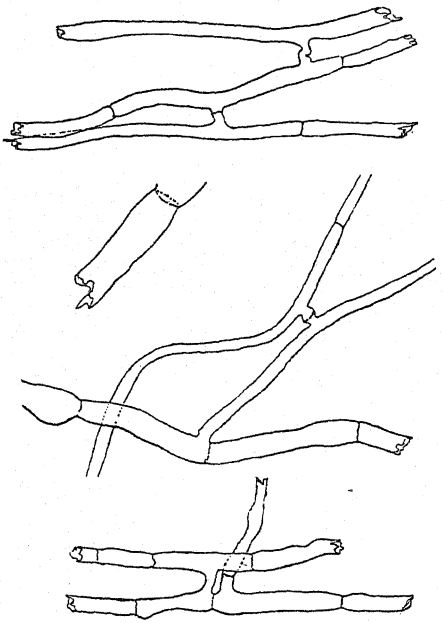


FIG. 6. H-connexions between hyphae of *Phoma* sp. in artificial media (Strain from Ettersburg 121). × 670.

the substratum. At the limit of the agar, against the walls of the tube, some very few slender, loose, cottony hyphae develop aerially. In pure cultures dark brown pycnidia are produced in great profusion.

DESCRIPTION OF THE FUNGUS IN CULTURE.

In culture the hyphae are very irregular, crooked, highly branched, varying in diameter from 2 to 15 μ, the larger portions septate, thick-walled, often constricted at the septa so as to form vesicular structures which are vacuolate and give rise abruptly either terminally or laterally to slender, non-septate branches (Fig. 5 *c*). This variation in diameter of the hyphae is so striking that at first there was some doubt as to the purity of the culture in question. However, the two types in many instances have been found conjugating by characteristic H-connexions (Fig. 6), which leaves no doubt as to their belonging to the same strain. Vesicles of the type figured by Rayner (18) are rare on the pycnidial strains, but occur in profusion on some of the other strains. The pycnidia are initiated by

a tangling together of two branches from large hyphae (Fig. 5, *a*). When mature they are nearly spherical, 100 to 200 μ in diameter, pale yellow-brown, smooth, often with two ostioles, slightly papillate at the mouth. The spores are many, unicellular, slightly falcate and blunt at the ends, 4 to 6 μ long by 2 to 3 μ wide, mostly without oil droplets. The above description is of a strain isolated from the horticultural variety Ettersburg 121. The pycnidia of strains from horticultural variety Howard 17 and from *F. platypetala* vary somewhat from this in colour and diameter. Strains which have not yet borne pycnidia have been isolated from all of the other species and varieties of strawberry examined.

Discussion.

As was said in the beginning of this paper, certain of the strawberries, *F. Chiloensis* in particular, have long presented a puzzle to horticulturists as regards their distribution and special requirements. In view of these facts the isolation of several apparently distinct strains of *Phoma* from different forms of strawberries takes on considerable interest. It is to be recalled that *Phoma radicis* is the fungus which both Ternetz (22) and Rayner (18, 19) have isolated from the mycorrhizal Ericaceae such as those known to be commonly associated with *F. Chiloensis*, and it is with this fungus that the latter writer has done probably the most conclusive work yet published on the synthesis of mycorrhiza under controlled conditions. The behaviour of the fungus found in *Fragaria* is certainly very similar to that described by Rayner (18–20) and Gallaud (5), and although artificial synthesis of mycorrhiza in sterilely-grown seedlings will be necessary before we can be quite certain of the relationships involved, the evidence for a mycorrhizal relation so far appears good. Certainly ecological studies and further cultural work in the laboratory seem likely to clear up some of the puzzling points in the behaviour of the strawberry.

Summary.

1. Certain peculiarities of the Chilean strawberry *F. Chiloensis*, particularly as to distribution, have long been puzzling to horticulturists, and the importance of this species has made desirable an analysis of these peculiarities.

2. A typical arbusculate fungus having the appearance of a mycorrhizal growth has been found in the roots of this and other species of strawberry, and suggests an explanation of these anomalous characters.

3. Attempts to isolate this mycorrhizal fungus have resulted in the separation of several fungi, among them a *Phoma* (one or more?), which appears to be the fungus in question.

4. Further work on the artificial synthesis of mycorrhiza from pure

cultures will be necessary before the real nature of this association, and its importance in horticulture can be expressed with certainty. Careful ecological studies should also be made.

COLUMBIA, MISSOURI.
October 30, 1928.

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Spermatogenesis and Fertilization in the Cleistogamous Flower of Viola odorata, var. praecox, Gregory.

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INTRODUCTION.

ALTHOUGH for many years cleistogamy in *Viola* has been observed, and the various forms of flower produced have been examined, it has never been known whether fertilization occurred. It was with a view to

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solving this problem that the present investigation was undertaken, and it has served to show that at least in the case of *Viola odorata* var. *praecox*, G. the seeds produced by cleistogamous flowers are the outcome of a true fertilization.

Most of the descriptions of cleistogamous flowers are to be found in the literature of the last century, by workers concerned with the question of the origin of cleistogamy. D. Müller (13), in 1857, made the first observations on *Viola*, and he described the cleistogamous flowers of about six species. Mohl corrected some of the statements that Müller had made, and later accounts really add nothing essential to the description given by these two botanists. In 1904 and 1905, Goebel (4, 5) collected all the evidence that had been previously recorded with regard to cleistogamous flower formation, and showed that the cleistogamous flower is a flower arrested in its development, although the sexual organs have matured; opposing Darwin's theory of a special modification in its structure. Goebel also tried to prove that cleistogamous flowers are formed as a result of the plant receiving insufficient nourishment. The accounts are in many ways incomplete and partly incorrect; in many cases the variety of the species is not given, and even the attempts made to 'explain' cleistogamous flowers result in no uniform picture. It has therefore been considered advisable to give an exact account of the occurrence of cleistogamous flowers in this variety; together with a brief comparison of the three types of flower formed.

This investigation, therefore, includes observations on the normal yearly cycle of flower production in this variety; a comparison of chasmogamous, cleistogamous, and the so-called semi-cleistogamous flowers, together with a more detailed account of the cytology of the cleistogamous flower, with special reference to the fertilization stage.

Material and Method.

Material. Most of the material has been grown in two beds in the Royal Holloway College Botanical Garden, where plants have been kept under continual observation. One bed is exposed to full sunlight, the other is in the shade. At first many species were examined, which were obtained from the Royal Botanic Gardens at Kew, the Chelsea Physic Garden, and the Royal Horticultural Society's gardens at Wisley. The research ultimately narrowed down to one form, viz. *Viola odorata* var. *praecox*, G. I am indebted to Dr. Eric Drabble, to whom I was referred by the keeper of the Herbarium at Kew, for its identification. This plant was chosen because of its continuous flowering throughout the year, the abundant formation of semi-cleistogamous flowers, and the fact that the chasmogamous flowers never set seed.

Method. Continuous observation of the flowering cycle of plants of this variety have been made in both sun and shade beds.

Form and structure of flowers have been elucidated by dissection of living material, with measurements of the relative size and position of different parts, while the germination of pollen-grains of different sizes and shapes has been tested in hanging drop cultures of various solutions.

For special anatomy and cytology, material has been fixed at many different times of the day, in as many different stages of development as possible, in the following fixatives: Alcohols 50 per cent., 70 per cent., 90 per cent.; Acetic Alcohol (Farmer's fluid); Bouin's fluid; Allen's Modification of Bouin's fluid; Chromacetic (medium); Flemming's fluid (strong); Hermann's fluid; Merkel's fluid. The tips of the sepals and petals were cut off, and the base of the ovary cut through in order to allow easy penetration of the tissues by the fixing fluid, and in most cases an exhaust pump was employed. After clearing in xylol and embedding in paraffin-wax, sections have been cut from 6 μ to 10 μ in thickness, and stained with various combinations of stains, including—Aniline safranin and licht grün; Heidenhain's iron-alum haematoxylin with various counter-stains; Flemming's triple stain; Breinl stain. The most successful results have been obtained from material fixed in Allen's modification of Bouin's fluid, and stained in Heidenhain's iron-alum haematoxylin, with or without counterstain.

THE FLOWERING CYCLE OF *V. ODORATA* VAR. *PRAECOX*, G.

On the plants under observation during the two years' research the flowers have been borne as follows:—

September 1924 to March 1925,	chasmogamous flowers;
April 1925 to May 1925,	semi-cleistogamous flowers;
May 1925 to September 1925,	cleistogamous flowers;
Mid-September 1925,	semi-cleistogamous flowers;
Late September 1925 to March 1926,	chasmogamous flowers;
April 1926 and May 1926,	semi-cleistogamous flowers;
May 1926 to July 1926,	cleistogamous flowers.

Thus it appears that flowers of one kind or another are to be found in this form at every season of the year, except that for an interval of about a week, at the time when one form is giving way to another, only young buds are to be found. The semi-cleistogamous flowers mentioned above were produced by plants of *Viola odorata* in both sun and shade beds at Royal Holloway College after normal flowering had ceased. They are intermediate in form between chasmogamous and cleistogamous flowers, and are therefore presumed to be the semi-cleistogamous flowers mentioned by Knüth and Goebel. No difference in the flowering periods was noticed in the sun and shade plants, though the shade bed produced a relatively large number of chasmogamous flowers, while the sun bed produced more cleistogamous flowers.

A Comparative Description of Chasmogamous, Cleistogamous, and Semi-cleistogamous Flowers.[1]

The flowers have been compared from the point of view of the relationship of parts, especially the relative position of stigma and anthers; and from the point of view of a peculiar tendency of the pollen-grain to germinate within the anther, thus rendering pollen dispersal impossible; of variation in size, shape, contents, and germinating power of the pollen-grains; and, lastly, of seed formation.

Chasmogamous Flowers.

Description of Parts.

Calyx. Five free sepals, green, tinged violet, ovate, 6–7 mm. × 2·5 mm., the two anterior separated by a petal spur, and all five with basal flap extensions, 1·5 mm. long, which together form an investment round the base of the flower.

Corolla. Five free petals, alternating with the sepals; the anterior petal produced into a relatively long hollow spur. Two posterior petals 10 mm. × 6 mm., two lateral petals 11 mm. × 6 mm., anterior petal 16 mm. × 8 mm. (including spur), spur 4–8 mm. long and compressed in the vertical plane.

Androecium. Five free stamens, alternating with the petals; filaments negligible; anthers introse, 2 mm. × 1·5 mm., cream in colour, with connective produced into a triangular orange flap, 2 mm. long. The whole staminal group constitutes a cone around the gynaecium, 4 mm. high and 3 mm. in diameter, whose apex is formed by the orange flaps closely pressed against the style. The two stamens inside the anterior petal possess nectary tails which converge into the petal spur as green, blunt, compressed processes, 3 mm. in length.

Gynaecium. Syncarpous of three carpels; ovary, green, conoid, 2·5 mm. long, enclosed by anthers; cavity unilocular, with three parietal placentas bearing anatropous ovules in two or three rows, total number of ovules averaging thirty. The style extends about 1·5 mm. above the connective flap of the stamens, is hooked at the end (1 mm.), the point being bent over the spurred petal, and slightly papillose at the tip. The ovary produces simple hairs on its upper surface, where exposed between the connective flaps, these hairs continuing down the ovary wall in more or less well-marked lines.

Nectary. The tailed processes of the two front stamens secrete nectar,

[1] The description of *Viola odorata* in 'Types of Floral Mechanism', by H. A. Church, has been used as the basis of the description for this variety *praecox*.

and the spur of the anterior petal acts both as a protective sheath and a storage reservoir.

An examination of these flowers during the autumn of 1924 revealed three unusual characters. Firstly, all the anthers of the open chasmogamous flowers contained pollen-grains already germinating in masses. This germination had begun even before the flowers opened, and no dehiscence of the pollen-sacs occurred. Secondly, in buds where the pollen had not yet started to germinate, the pollen-grains varied in shape, size, and contents. Thirdly, none of these flowers set seed. Soon after opening they shrivelled up and later rotted away. An investigation of these chasmogamous flowers was therefore continued throughout both flowering seasons. The plants, from which the investigated flowers were obtained, bore flowers freely during both winters. During January and February the number and size of the flowers steadily decreased.

Germination of the Pollen-grains within the Anthers.

In every open flower examined, some pollen-grains were found germinating within the anthers, but the proportion varied. From December to March each year a gradual decrease was recorded in the amount of pollen which germinated *in situ* within the anther. Records made in December and January show that in open flowers the pollen was germinating in such masses that individual pollen-grains could not be distinguished. In February only a small percentage of the pollen inside the anthers was germinating, so that the size and shape of the pollen-grains in the anthers of open flowers could be distinguished clearly for the first time. At this stage the anther wall may partly break along the line of dehiscence. Most of the pollen-tubes remain in a tangled mass within the anther. This is difficult to disentangle, but there are always some pollen-tubes that have made their way through the anther wall. These grow in any direction, and do not attain a great length. When pollen-tubes can be separated from the tangled mass inside the anther, the growth is seen to be rather peculiar. A short portion at the tip of the pollen-tube is about twice as thick as the rest, and the pollen-tube, instead of growing more or less straight, is very sinuous and sometimes twisted like a corkscrew.

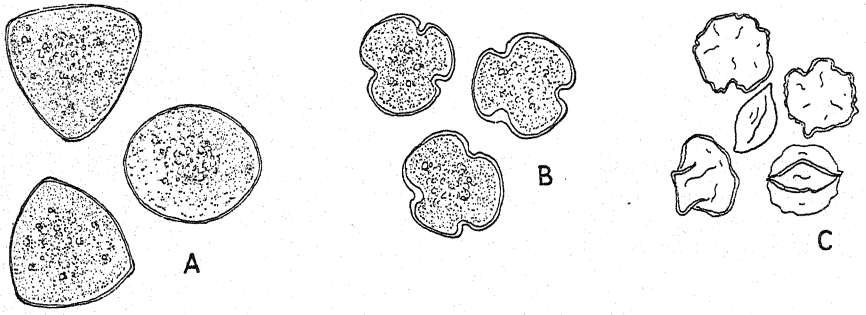
Variation in Pollen-grains.

Three forms of pollen are distinguishable in the anthers of chasmogamous flowers. Sometimes all three occur in one anther, at other times only two forms can be found. The differences are in shape, size, starch content, and germinating power. Although many flowers have been examined, no attempt has been made to draw any definite conclusion as to the cause of these various forms, or the regularity with which they occur.

Shape and Size. Text-fig. 1 illustrates the three types of pollen-grain found.

I. Large tetrahedral or ovoid pollen-grains, with very thin walls; the walls slightly differentiated at the angles by additional thickening. The protoplasm becomes vacuolate before germination begins.

II. Rounded, ridged pollen-grains, slightly smaller than those described above, with three longitudinal edges. The wall is thicker and more transparent, with additional cellulose thickening in the grooves. The protoplasm is less vacuolate than in I.



TEXT-FIG. 1. Pollen-grains from bud of chasmogamic flower. (1) Ovoid and tetrahedral; (2) round and ridged; (3) shrivelled.

III. Shrivelled pollen-grains with thick walls which are grooved as in II. These are empty.

Starch Content. The starch content of these pollen-grains is not a constant character. Starch may occur in Forms I and II, and is found much more frequently in pollen-grains taken from flowers borne in November and December. Towards the end of the flowering period the starch content becomes less, and no starch has been found in the flowers borne in February. At the same time a tendency towards a less sharp distinction between the three forms of pollen-grain is noticeable. In February and March pollen-grains intermediate between the first two kinds have been found.

Germinating Power. The germinating power of these pollen-grains varies. This has been tested in hanging drop cultures in water, or dilute solutions of cane sugar. Pollen-grains of all three kinds were taken from large buds at a stage just before germination, and placed in the hanging drop and left for several hours. In every case the large tetrahedral or ovoid pollen-grains germinated after about four to six hours, putting out straight pollen-tubes which grew to a length of six or seven times their own width, and still appeared vigorous. The other grains could not be induced to germinate.

Power of Setting Seed.

In no case did any of the chasmogamous flowers set seed, but all rotted away soon after they had opened. Even should pollen-tubes, which have grown through the anther wall, still retain their fertility, yet the

position of the anthers with regard to the stigma in the chasmogamous flower is such that the pollen-tubes do not reach the stigma, and self-fertilization cannot take place (Text-fig. 4). On the other hand cross-fertilization is unlikely, since pollen-grains are not shed, and there is no cross-pollination.

Cleistogamous Flowers.

Description of Parts.

Calyx. Five free sepals, three of which are wider than the others, violet, green, ovate, 6 mm. × 1·5 mm. and 6 mm. × 1 mm.; all five with basal flap extensions 1 mm. long, which together form an investment round the base of the flower. The sepals form a cone completely enclosing the flower. Two or more of the sepals have glands in the edge near the base.

Corolla. Five rudimentary petals, nearly white in colour, varying in size, but all very small, about 2 mm. long. There is no sign of a spur. Occasionally differences in numbers are observed and flowers with four or six petals found.

Androecium. Five free stamens, alternating with the petals; filaments comparatively longer than in the chasmogamous flower; anthers 1·5 mm. long, possessing a small rounded orange connective flap which is about one-sixth of the total length of the stamen. The whole staminal group completely enclose the style and stigma, forming a hood over them. There are no signs of nectary tails. Occasional abnormalities are observed when two stamens are larger than the other three, or when one or more of the stamens possess only one rudimentary pollen-sac.

Gynaecium. Syncarpous of three carpels; ovary green, spherical, unilocular, with three parietal placentas bearing fewer ovules than the chasmogamous flower; style very short and bent, so that the stigmatic surface is brought very near one of the anthers; simple hairs borne more or less uniformly over the ovary.

Germination of the Pollen-grains within the Anthers.

All the anthers of mature flowers contain pollen-grains which germinate freely within the anther. Nearly all of these pollen-tubes make their way directly to the top of the pollen-sac, where they push through the wall and grow in the direction of the stigma. When a number of tubes have penetrated the wall at this place they twine round each other as they grow, so that a thick, many-stranded rope of pollen-tubes stretches from the anther wall to the stigma. At this stage it is almost impossible to pull the anthers away from the stigma. Attempts at dissection usually result in a portion of the stigmatic surface being torn away with the anthers.

Variation in Size, Shape, and Contents of the Pollen-grains.

In the cleistogamous, as well as in the chasmogamous flowers, variation in pollen-grains is found. Two forms are distinguishable, sometimes both occur in one anther, at other times only one form can be found. The differences are in shape, size, starch content, and germinating power.

Shape and Size. Text-fig. 5, illustrates the two types of pollen-grain found.

I. Large tetrahedral or ovoid pollen-grains, with thin cellulose wall. No differentation of thickening can be seen. These are few in number. (Text-fig. 5 (1)).

II. Rounded ridged pollen-grains slightly smaller than those described above, with three longitudinal ridges; wall much thicker and more transparent with additional thickening in the grooves. (Text-fig. 5 (2)).

Starch Content. Starch is usually found in the second form of pollen described, and only very rarely in the first form. Here, as in the pollen-grains of chasmogamous flowers, it is not a constant character.

Germinating Power. The germinating power of these pollen-grains has been tested in hanging drop cultures in water or dilute solutions of cane sugar. Pollen of the first form cannot be induced to germinate, while pollen of the second form germinates freely, putting out straight pollen-tubes which grow to a length of six or seven times their own width.

These facts are of interest when considered in connexion with the cytology of the pollen-grain, described below. A stained section through the ovoid pollen-grains shows that this is the type that possesses abnormal nuclei. It is significant that these pollen-grains cannot be made to germinate outside the anther, although they are found occasionally with short tubes inside the anther. It is also significant that in the cleistogamous flower it is the second type of pollen-grain which germinates, while in the chasmogamous flower it is the tetrahedral or ovoid pollen-grains which put out long tubes in hanging drop cultures.

Power of the Cleistogamous Flowers to set Seed.

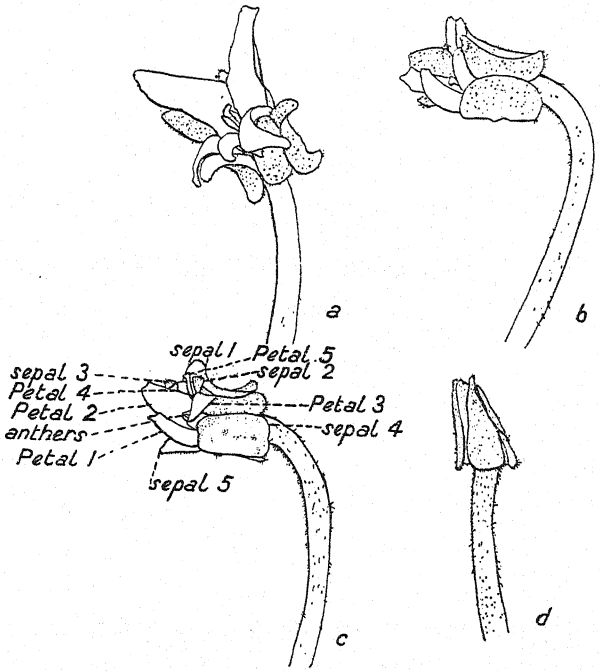
All these flowers set seed freely.

Semi-cleistogamous Flowers.

It has been found possible to give a precise description, both of a chasmogamous and of a cleistogamous flower. In the case of the semi-cleistogamous flowers this cannot be done as so many variations in form and size are found, and more than one type of semi-cleistogamous flower must be described.

Towards the end of March it was noticed that the chasmogamous flowers were smaller than those which had been produced in January and

February, but the formation of semi-cleistogamous flowers in April introduced a very definite stage towards the development of true cleistogamy. These flowers differ from the normal flowers in several morphological characters. The size of the whole flower and of its parts is much reduced.

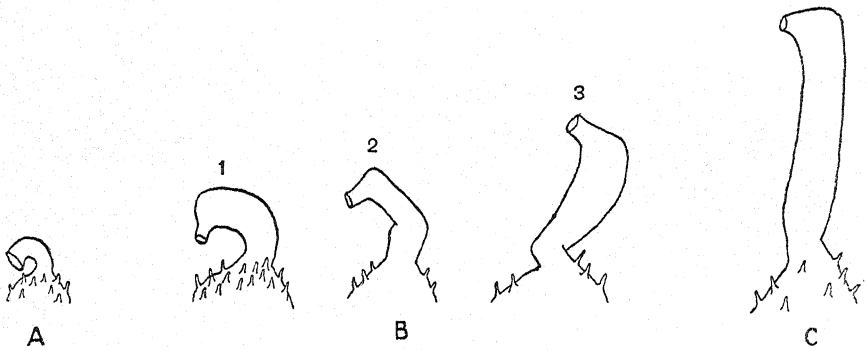


TEXT-FIG. 2. a–c. Semi-cleistogamous. d. Cleistogamous flowers.

There is further bending and shortening of the style which brings the stigma closer to the ovary wall and more in touch with the stamens. There is complete absence in all these flowers of both the spur and the two nectaries. There is greater regularity in the pollen-grains of the first formed semi-cleistogamous flowers. The buds from which the semi-cleistogamous flowers are developed resemble cleistogamous flowers. They are produced in the axils of leaves borne on runners, rarely in the axils of leaves forming the crown of the parent plant, although buds of chasmogamous flowers are produced here. The buds are much more pointed and the pair of bracts arises some way down the stalk of the bud instead of immediately below it.

In the case of the first two differences enumerated, namely reduction in size and bending of the style, a gradual change occurs, so that during the time they are forming the flowers become smaller and the style becomes more and more bent, although at any one time a whole range of such stages may be found in any one plant. (Text-fig. 3). With regard to the occurrence of spur and nectaries and the form of the bud, no gradual

change occurs, so that all semi-cleistogamous flowers resemble cleistogamous ones in that they possess the same form of bud, and neither spur nor nectaries. Hence it cannot be said that semi-cleistogamous flowers show every stage intermediate between chasmogamous and cleistogamous flowers, rather does their appearance introduce a definite stage towards the development of true cleistogamy. Nevertheless three distinct forms of this semi-cleistogamous flower can be recognized, differing chiefly with regard to method of pollination. These are described below as three types.



TEXT-FIG. 3. A–C. Stigma, style, and part of ovary of the three types of flowers to show progressive bending of the style. A. Cleistogamous. B. Semi-cleistogamous. C. Chasmogamous.

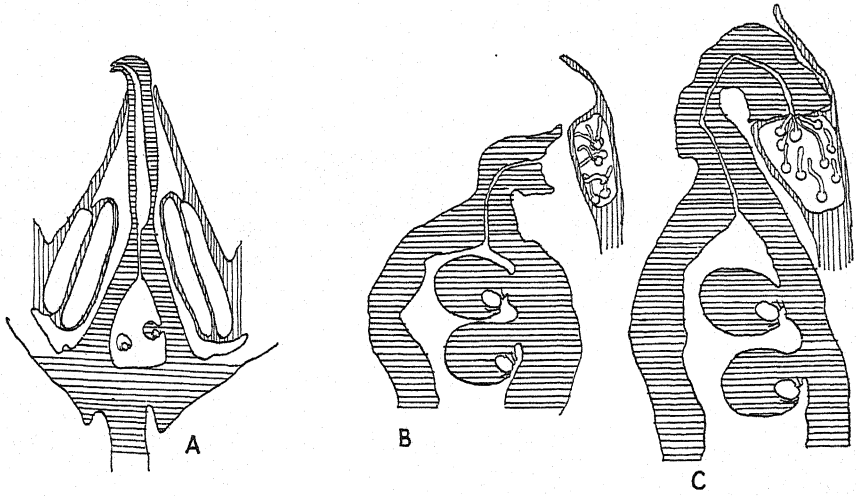
Type I.

In the earliest semi-cleistogamous flowers the anthers dehisce so that the pollen is shed and none of it germinates within the anther. This is of interest in that the last formed chasmogamous flowers contain very little pollen germinating *in situ* and the anthers show signs of dehiscence. Since the anthers are introse, the pollen is shed inwards on to the style and stigma, which, as the style is bent and much shorter than in the chasmogamous flower, is in such a position as to receive it. Thus seed was set in April, 1925, for the first time since September, 1924. The fact that self-pollination and not cross-pollination occurred seems undeniable from the fact that in most cases the stigma was entirely hidden by the connective of the anthers so that no outside pollen could reach it. At this stage the pollen is regular, and all of the form described in the chasmogamous flowers as round and ridged. The walls of the pollen-grain are thick and there is no starch content. This is the only time in the yearly cycle of flower production that true pollination (the placing of pollen on the stigma) occurs.

Type II.

The first stage is quickly passed over and, in later formed semi-cleistogamous flowers, germination of the pollen-grains begins to take place *in situ*. Germination is at first infrequent and a stage is passed through in which the

anthers still dehisce introsely so that some of the pollen is shed, while a few pollen-tubes are seen germinating through the top of the pollen-sacs. The pollen in this case is not so regular, and at least three forms can be recognized: grains of the form found in Type I which are rounded, grooved, with very thick walls and possessing no starch content; similar grains possessing



TEXT-FIG. 4. A–C. A. Longitudinal section of the bud of the chasmogamous flower of *V. odorata*. B. Longitudinal section of ovary and anther of the cleistogamous flower of *V. odorata*. C. Similar to B but of *V. sylvatica*.

starch, rather infrequent; and thin walled oval pollen-grains very densely filled with starch. It seems that pollen-grains of the first form germinate inside the anther, while the other forms are shed or remain ungerminated inside it.

Type III.

In semi-cleistogamous flowers developed still later, the pollen-grains germinate to a much larger extent, and the pollen-sacs no longer dehisce. Many of the pollen-tubes pierce the wall of the pollen-sacs, and grow out on to the stigma. It occasionally happened that in flowers in which the style was not much bent seeds were not set: the stigma was out of reach of the pollen-tubes (Text-fig. 3 (B3)). But this is unusual. Flowers at this stage possess pollen-grains of the three forms found in flowers of Type II.

Thus the success of the cleistogamous flower in setting seed seems to be determined chiefly by three phenomena.

1. Power of the pollen to germinate within the anther.

2. Power of the pollen-tubes to penetrate the anther wall.

3. The relative positions of anthers and stigma whereby it is made possible for the pollen-tubes to reach the stigmatic surface.

It is significant to note that the first two of these phenomena are precisely those which have been cited above as the cause of the loss of seed production in the chasmogamous flower. Coupled with the third character they ensure complete success in the cleistogamous flower. It should be remembered, too, that the pollen-grains which germinate within the anther of cleistogamous flowers are not of the same form as those which germinate within the anther of chasmogamous flowers.

In *Viola odorata* the mechanism is not so exact as in *Viola sylvestris* where two anthers become specialized, and are interlocked by means of peg-like outgrowths on the filament to form a hood which exactly fits over the top of the style and the stigma. One of these anthers is tightly pressed against the stigmatic surface. The pollen-tubes all emerge from the pollen-sac at that place where dehiscence would occur if the pollen were shed, and grow right down the stylar canal. In *Viola odorata* the pollen-tubes pierce the anther wall at several places near the top of the anther. The five anthers are locked together over the bent style, but not one of them is tightly pressed against the stigmatic surface.

Cytology of the Cleistogamous Flower.

Development of Pollen.

Pollen Mother-cells.

The early stages of pollen development appear to be quite regular. The number of pollen mother-cells found in each pollen-sac is fewer than in the chasmogamous flowers and varies in number in the different anthers of one flower. In certain phases of the reduction division the haploid number of *ten* chromosomes has been repeatedly counted. In early prophase the pollen mother-cells are packed tightly together and chromatin appears to be extruded from one into the other; by diakinesis they have rounded themselves off and are quite separate. The two daughter nuclei formed as a result of this division pass very quickly through interkinesis and the homotype division begins. This division is a rapid one and phases of it are much more rarely found than those of the heterotype division. The two spindles are very long and narrow and lie in two planes at right angles to each other. At the end of this division four free nuclei are found within each pollen mother-cell. It often happens that two or three of the pollen mother-cells in each pollen-sac are very late in development so that while the rest of the cells are in the four-nucleate stage, these cells are still in reduction phases. When the four nuclei are reorganized after the homotype division, the spindles remain connecting the nuclei and four additional ones are initiated, joining up all the nuclei and forming a tetrahedral figure. Wall formation now takes place; a plate is laid down across each spindle

and these join up so that a tetrad or four tightly pressed cells is found inside the pollen mother-cell wall.

Uninucleate Pollen.

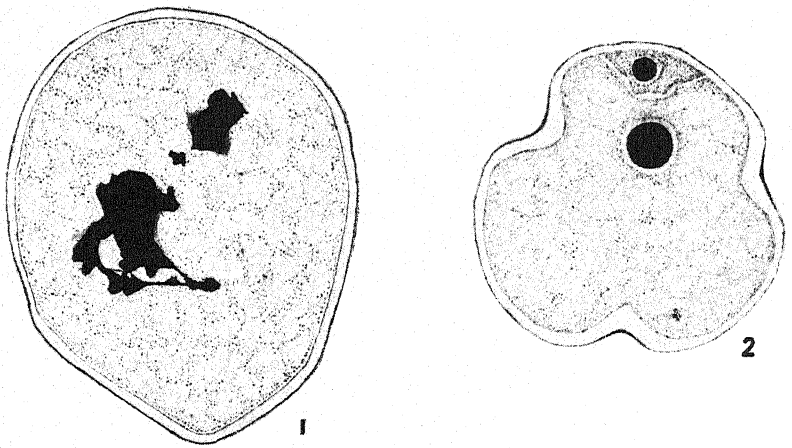
After a time the uninucleate pollen-grains break apart. At first these have a very irregular outline, being unevenly lobed. Each grain has dense protoplasm and a large nucleus. Young anthers have been found containing in the same sac both pollen mother-cells in the four-nucleate stage and uninucleate pollen-grains. The former are undersized and appear to be degenerating, and may have been formed from the backward mother-cells already mentioned. About this time occasional pollen-sacs are found in which the walls of the pollen-grains are replaced by numerous darkly-staining globules, which seem to be derived from wall substance. The entire tapetal layer in the pollen-sacs in which this type of disintegration is occurring, is frequently represented only by a mass of these spherical bodies. Latter (9) describes a similar condition in her paper on 'Pollen Development of *Lathyrus odoratus*', where she records such degeneration at an earlier stage.

Generative and Tube Nucleus.

The uninucleate pollen-grain grows in size and a large vacuole appears in the middle of the cell so that all the protoplasm is spread round the periphery. At one side it is thicker and has the nucleus embedded in it. This is now in a resting condition and is not quite so large as formerly. The pollen-grain continues to grow in size until it becomes approximately spherical. During this time the nucleus enlarges and begins to divide. This division is a very rapid one and stages are not frequently found. When division begins the protoplasm is still spread round the periphery and the nucleus is still very near the cell wall. The metaphase shows an unusually wide equatorial plate and short spindle. As division proceeds the protoplasm begins to fill once more the interior of the cell, encroaching from the side where nuclear division is taking place. While the two daughter nuclei resulting from this division pass into a resting condition, the cell wall begins to thicken and to assume the three-grooved form of the mature pollen-grain. The pollen-grain is now filled with rather vacuolate protoplasm. While the protoplasm immediately surrounding each nucleus is denser than the rest, that round the generative nucleus is organized into a definite cell. The two nuclei, both consisting almost entirely of nucleolus, always show a big difference in size. The larger one, which occupies the more central position in the pollen-grain, is the tube nucleus. The smaller one is the generative nucleus and is often only about one-third the size of the other and is more active in appearance. The generative cell in some cases appears to be pressed against the wall (Text-fig. 5 (2)).

Abnormalities in Pollen-grain.

From the description given it is evident that the development of the pollen-grain proceeds with few irregularities until after the division which gives the tube and generative nucleus. Degeneration of nuclei may now occur at almost every stage of development. Just before or at the time of germination any of the following conditions may exist in different pollen-sacs:



TEXT-FIG. 5. 1–2. The two types of pollen-grains found in the cleistogamous flower. 1. Abnormal and degenerating. 2. Normal with two nuclei.

1. Both nuclei in every pollen-grain are normal, and some of the pollen-grains are beginning to germinate in a perfectly regular manner. All these pollen-grains are the round ridged type described above in Text-fig. 5 (2).

2. Both nuclei in some of the pollen-grains appear abnormal, with the chromatin very deeply stainable and all structure apparently lost. These are of the ovoid type described above in Text-fig. 5 (1). They are always in the centre of the sac. A few of these grains possess short germ-tubes. In the same sac there are also to be found pollen-grains of the ovoid ridged type. These always line the anther wall and have therefore been nearest the tapetum. They have usually begun to germinate.

3. The tube and generative nucleus of some of the pollen-grains are large and have a pronounced and lumpy network which assumes a straggling outline. The grains are of an intermediate shape, and the nuclei are probably beginning to degenerate. These are in the centre of the sac. Some round ridged pollen-grains, usually germinating, are always to be found in the same sac next the tapetum.

Since it is only in condition 1 that *all* the pollen-grains in any one sac

are apparently alike, it may perhaps be assumed that conditions 2 and 3 are later stages of development arising from it, condition 2 being the last stage. This is supported by the fact that although the anthers may differ in the *amount* of degeneration among their pollen-grains, yet some pollen-grains of

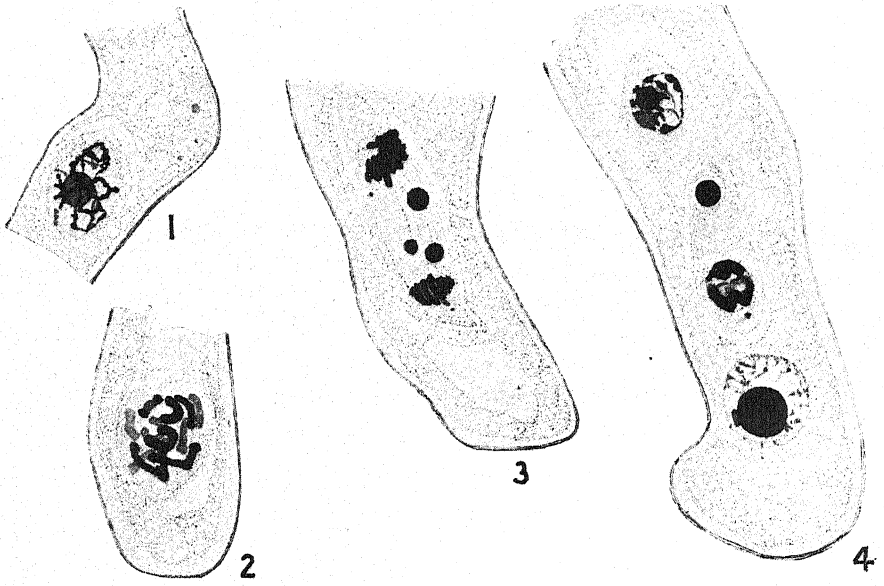


TEXT-FIG. 6. A germinated pollen-grain, of the type shown in 5 (2), showing the division of the generative nucleus and the degenerating tube nucleus.

the round ridged type, germinating in a regular manner, are always to be found in pollen-sacs along with the degenerating ovoid grains. Even at the time when division of the generative nucleus is taking place in some of the functioning pollen-tubes, numbers of ovoid degenerating pollen-grains can be found, some still ungerminated, others with short empty pollen-tubes. This question will be more fully dealt with in the discussion at the end of the paper.

Germination of Pollen and Division of Generative Cell.

It will be remembered that it was the round ridged type of pollen-grain that was found to germinate in the cleistogamous flower. The development of this grain proceeds rapidly. The germ-tube pushes its way through one of the grooves, where the wall, although thicker, appears to be of a more



TEXT-FIG. 7. 1–4. Stages in the division of the generative nucleus. 1. Thickened spireme. 2. Showing the chromosomes. 3. Telophase with globules of chromatin on the spindle. 4. Reorganization of the daughter nuclei and formation of male cells.

mucilaginous nature than elsewhere. The two nuclei travel towards the tip of the pollen-tube, and the generative nucleus begins to divide before the pollen-tube is very long, and always before the pollen-tube makes its way through the anther wall (Text-fig. 6). Quite often it is very near the tube nucleus when it begins to divide. In the prophase of the division the thread always appears deeply-staining and thick (Text-fig. 7 (1) and (2)). When the thread segments, the chromosomes are very long and *ten* can be counted (Text-fig. 7 (2)). The spindle is formed with its axis in the direction of growth of the pollen-tube (Text-fig. 7 (3) and (4)). As the generative cell divides, the specially differentiated protoplasm surrounding it becomes spindle-shaped so that it approximately coincides with the metaphase spindle (Text-fig. 6). The chromosomes arrange themselves on the equatorial plate. In the telophase of the division globules of chromatin are often found left behind on the spindle (Text-figs. 6 and 7 (3)). No suggestion of a cell-plate has been seen. As the two daughter nuclei are reorganized the protoplasm of the generative cell increases in bulk and

becomes divided into two so that a portion surrounds each of the two nuclei (Text-fig. 7 (4)). These are the two male cells and the protoplasm of each is now pear-shaped with the nucleus embedded in the larger end. The pointed end is like a tail and contains at least one globule of chromatin, which may be one of those left behind on the spindles of the previous division. The two cells lie one behind the other in the same plane as the spindle, with the two tails approaching each other and often overlapping and directed towards the centre. At this stage the male nuclei are spherical in shape and possess a definite reticulum. Small globules of chromatin are often found outside the male cells in the protoplasm of the pollen-tube itself (Text-fig. 7 (1) and 8). Although various stains have been used no suggestion of a wall round the male cells has been observed.

Meanwhile the tube nucleus may behave in any of the following ways:

1. It may still retain the form it possessed in the pollen-grain, although a fine reticulum is now seen (Text-fig. 7 (4)).

2. It may elongate so that its length is about four times its width, and show the lumpy network mentioned above.

3. It may degenerate in the pollen-tube while the generative nucleus is still dividing, in which case it is often found in the tip of the pollen-tube.

4. It may degenerate at a later stage, after the division of the generative nucleus, when it often appears to be cleft in two.

Soon after the division of the generative cell is completed, the pollen-tube pushes its way through the anther wall and grows towards the stigma. The male nuclei may behave in any of four ways.

1. They may degenerate while the pollen-tube is still within the anther.

2. They may degenerate while the pollen-tube is growing towards the stigma.

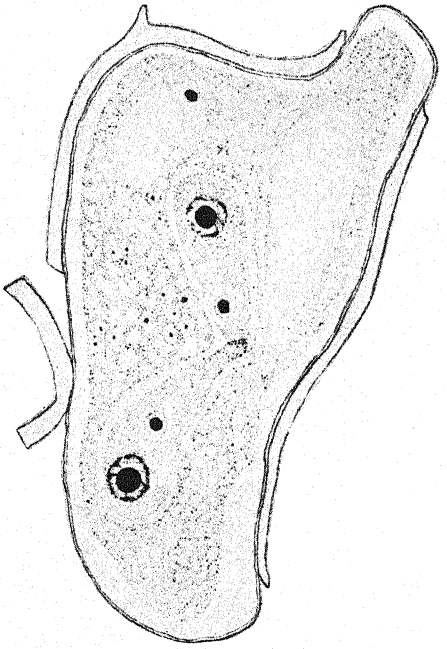
3. They may degenerate while the pollen-tube is in the stylar canal.

4. They may remain normal so that quite a number of pollen-tubes reach the ovules in a fertile condition. Pl. XII, Fig. 1, shows two normal male nuclei in the pollen-tube as it passes down the stylar canal. Frequently, however, the pollen-tube stains deeply at this stage so that it is impossible to distinguish the male cells till they pass out of the pollen-tube. By this time, the male nuclei have become ovoid in form instead of spherical as when first formed (Text-fig. 7 (4) and 8), and the protoplasm is now ovoid instead of pear-shaped, while the male cells do not overlap and no longer possess a tail.

If the tube nucleus remains regular in shape and size until the pollen-tube reaches the stigma, it begins to elongate and to form globules of chromatin as the pollen-tube passes down the stylar canal (Pl. XII, Fig. 1). Some of these no doubt are the globules which later surround the fertilized egg (Pl. XII, Fig. 2).

The occurrence of male cells, other than free nuclei, in the pollen-tube

is now found to be of more frequent occurrence than was formerly supposed. In all recent work on fertilization in angiosperms, almost without exception, the occurrence of male cells in the pollen-tube is reported. Ishikawa (7) on *Œnothera*, Wylie (22) on *Vallisneria*, Finn (3) on *Asclepias cornuti*, Welsford (20) on *Lilium auratum*, all agree in this respect. It is in the earlier



TEXT-FIG. 8. A germinated pollen-grain containing two male cells, with a globule of chromatin in the tail of each.

work that no mention of male cells is made. Schaffner (18) on *Sagittaria* and Merrell (11) on *Silphium* refer to the male gametes as though they were free nuclei and not complete cells.

Corresponding Stages in the Chasmogamous Flower.

The cytology of the corresponding stages in the chasmogamous flower has been followed merely with a view to comparing them with those just described for the cleistogamous flower. More abnormalities seem to occur in the development of the pollen. A greater amount of chromatin extrusion takes place in the pollen mother-cells, so that in succeeding stages the protoplasm has many small globules of deeply staining material scattered in it. Frequently, in the tetrad stage, one of the four nuclei becomes very deeply staining and elongated, until it finally degenerates. Many instances are found of the condition described for cleistogamous flowers where the

walls of the pollen mother-cells or pollen-grains are replaced by numerous darkly-staining globules which seem to be derived from wall substance. Many mature pollen-grains are found with degenerating nuclei as described for cleistogamous flowers. Others germinate in great profusion within the anther, and although the division of the generative cell has not been studied, yet definite male cells are occasionally seen to be formed within these pollen-tubes, showing that development may proceed at least up to this stage. Many of these pollen-tubes remain within the anther wall and none of them can function.

Development of Embryo Sac.

Megaspore Mother-cell.

At first the young ovule consists of a more or less spherical mass of undifferentiated tissue. No attempt has been made to trace the origin of the archesporial tissue, but Bliss in the chasmogamous flower of *Viola odorata* shows that it arises from a single cell. At a later stage about eight archesporial cells stand out clearly in the middle of the ovule, by reason of their greater size and larger nuclei, all dividing simultaneously. One of these cells becomes the megaspore mother-cell. At the same time the two integuments begin to develop by repeated divisions of some of the cells outside the archesporium. The megaspore mother-cell increases in size, and the nucleus enters into the prophase of the meiotic division. Detailed work on the phases of this division has not been made part of the present study, but diakinesis has been found repeatedly and the haploid number of *ten* chromosomes counted. The heterotype division is completed and a wall formed between the two daughter nuclei. The homotype division follows immediately. The spindle of each nucleus in this division lies obliquely across the cell from corner to corner, so that the two spindles are at right angles to each other.

Young Embryo Sac.

A row of four megaspores is formed. The one at the chalazal end enlarges to form the embryo sac while the other three rapidly degenerate. The young embryo sac continues to enlarge and its nucleus rapidly divides. The two daughter nuclei pass one to each end of the embryo sac and become separated by a large vacuole which fills the central portion of the cell. Each nucleus divides twice in quick succession so that four nuclei are formed at each end. One nucleus from each end passes into the centre of the embryo sac and the mature eight nucleate embryo sac is organized. In each division after meiosis the haploid number of chromosomes is seen. This makes it quite certain that the nuclei in the eight nucleate embryo sac remain haploid without any change in chromosome number after the reduction division.

Eight Nucleate Embryo Sac.

A mature embryo sac contains the egg, two synergids, two polar nuclei and three antipodal cells, typically arranged; synergids and egg are at the micropylar end of the sac, the three antipodals at the chalazal end, while the two polars are centrally placed.

The synergids are large and almost touching each other at their apical end (the part towards the micropyle) but a little apart at the base. On the free side each has an indentation. The apex forms the filiform apparatus, a solid mass of conical shape which is perforated by a number of minute canals which arise in the basal part and converge upwards. The nucleus is usually centrally placed and contains little stainable material outside the nucleolus. A large vacuole appears below the nucleus. In the rest of the cell the protoplasm is dense with few vacuoles. Previous to fertilization the synergid nuclei become a little larger and have a more active appearance in that traces of a reticulum are seen. The whole appearance closely resembles that described by Ishikawa for *Œnothera* (7).

The egg is pressed against one or both synergids so much so that there seems to be no definable membrane between the protoplasm of the egg and one synergid. They are practically continuous in a longitudinal section of the embryo sac. The egg is spherical in shape with a large vacuole which occupies more than half its volume, and which occurs in the side of the egg nearest the synergid. Thus in some sections the vacuole of the egg is separated from the vacuole of the synergid by such a thin layer of protoplasm that the vacuoles seem to be continuous, while the protoplasm of the egg appears crescent-shaped in section. In fact it could not be decided for some time whether the egg should be described as spherical with a large vacuole, or cup-shaped and fitting round the base of the synergid. In transverse sections, however, a more definite boundary can be seen. Certainly no distinct cellulose membrane exists between the synergids and the egg as described by Ishikawa for *Œnothera* (7). The egg nucleus is larger than the nuclei of the synergids. Practically all the stainable material is aggregated in the nucleolus and no definite reticulum can be demonstrated. The protoplasm has a finely granular appearance.

The two polar nuclei are usually embedded in a thick strand of protoplasm just below the egg. Here, as in the egg nucleus, practically all the stainable material is aggregated in the nucleoli, which are very large. Either no network or only a very faint suggestion of one is seen. The polar nuclei lie side by side and are still distinct at the time of fertilization. They are pressed against each other: sometimes separated by only a thin layer of protoplasm. The membrane of each nucleus is in some cases indistinct, making it difficult to decide the exact boundary of the nuclei which sometimes seem to merge gradually into protoplasm. This is the first sign of

preparation for the fusion of the two nuclei which is not carried any further until after the male nucleus arrives. More often the two nuclei remain absolutely distinct until the entry of the pollen-tube into the embryo sac. When they are very much de-stained, their nuclei are seen to be extraordinarily vacuolate, so that although perfectly spherical in shape when entire, they look like a sponge (Pl. XII, Fig. 8, B). So many vacuoles are there, that if the nucleolus be found cut, the smaller portion does not present an entire edge, but one irregularly scalloped.

The polar nucleoli do not de-stain gradually towards the interior as one would expect. There is a sharp contrast between a portion in the centre and the peripheral region: the central area remains very much stained and well defined, while the peripheral region is practically colourless. The same condition is shown after fertilization. This is due to differentiation in staining since all stages are found, from those in which the entire nucleolus is dark, to those where only a very small portion in the centre is left stained and definite. At the same time there must be a reason for the very sharp contrast often seen. Occasionally the same phenomena is observed in the egg nucleus.

The polar nuclei increase in size just before fertilization.

The antipodal cells are small and insignificant and very soon disappear.

Corresponding Stages in the Chasmogamous Flower.

The development of the embryo sac seems to resemble that already described for the cleistogamous flower, while the mature embryo sac is very like it. The embryo sac is larger, and the egg appears to be mature and ready for fertilization. All the nuclei are characterized by their large nucleoli and lack of stainable reticulum.

Fertilization and Endosperm Formation.

Entry of the Pollen-tube.

The pollen-tube passes down the micropyle and pushes its way through the nucellus, where it becomes wider and frequently branches or bulges. When it enters the embryo sac it passes right through one of the synergids, entering by the filiform apparatus at the apex. At the base of the synergid some of the contents are discharged on to the egg cell. Occasionally the pollen-tube appears to extend a little further below the synergid before it discharges, but more frequently its end appears level with the base of the synergid. The pollen-tube is filled at this stage with material stainable with chromatin stains so that even in very thin sections it has not been possible to distinguish the male-cells in the end of the pollen-tube either at, or preceding, the time of discharge. Part at least of the stainable material in the end of the pollen-tube must have come from the degeneration of the

tube nucleus. That part of the stainable material which is expelled by the discharging pollen-tube often occurs as globules. Many of these globules surround the egg, and for some time it was extremely difficult to decide whether the male cells were still in existence or whether they had degenerated and given rise to part of the stainable material found (Pl. XII, Figs. 2, 3, 4).

In all cases the end of the pollen-tube is pressed against the egg, while in certain cases it has been seen pushing into the egg cytoplasm so that its end was almost touching the membrane of the egg nucleus. It has been shown that the egg is usually pressed much more tightly against one of the synergids than against the other, and it is of interest that in every case observed the pollen-tube enters by the other synergid: the one which is not in such close association with the egg. Inside the synergid the pollen-tube stains very deeply with chromatin stains, so that the nucleus of the synergid cannot be seen. The other synergid remains large and active and does not degenerate until after the fertilized egg begins to divide. In no case has more than one pollen-tube been observed to enter the embryo sac.

The passage of the pollen-tube through one of the synergids does not seem to be an unusual occurrence in angiosperms. In this case, however, the pouring out of the contents of the pollen-tube into the synergid described by Ishikawa for *Œnothera* (7), and Guignard for *Nicotiana tabacum* and *Datura laevis* (6), does not occur. Evidently the pollen-tube remains intact until it reaches the base of the synergid, since in some cases when the synergid has been cut some of the unstained protoplasm of the synergid can be distinguished apart from the darkly stained contents of the pollen-tube. Probably the pollen-tube forces its way through, or dissolves away, the wall at the base of the synergid before it discharges its contents.

Male Nuclei.

Two male cells can be clearly demonstrated near the tip of the pollen-tube as it passes down the stylar canal (Pl. XII, Fig. 1). After this they are lost trace of, owing to the deeply stainable nature of the pollen-tube, until they are seen as two male nuclei, approaching respectively the egg and polar nuclei. Hence it cannot be said whether they are expelled from the pollen-tube as male cells which lose their protoplasmic sheath almost immediately, or whether the protoplasmic sheath is left behind in the pollen-tube so that at the actual moment of discharging of the pollen-tube the male gametes exist as free male nuclei. In a preparation of the earliest stage obtained after the pollen-tube had discharged, a small portion of dense cytoplasm is seen a little distance behind each of the male nuclei, which suggests from its position and appearance that it might be the protoplasmic sheath which has been shed after the emergence of the male cells from the pollen-tube. No definite statement can be made with regard to this point.

The male nuclei are of an ovoid shape and, unlike the other nuclei described, possess a definite reticulum, which is sometimes very granular, and a small nucleolus situated just inside the membrane. When approaching the egg and polar nuclei they are nearly the same size and shape as they were in the pollen-tube just before it reached the ovary, but have become less elongate and more nearly spherical (Pl. XII, Fig. 3). No suggestion of the vermiform shape described by Sax for *Frittilaria pudica* (17), and by Welsford for *Lilium auratum* (20), other than this slight elongation, has been found.

In existing records of fertilization in angiosperms, the male gametes have been described as behaving in one of three ways.

In some cases male cells do not appear to be formed ; the male gametes exist as free nuclei from the moment of their formation by the division of the generative cell, until they fuse with the egg and polar nuclei. This behaviour is described by Sawyer for *Iris versicolor* (16). Miss Bliss in her paper on the life history of *Viola* (2) does not find male cells in the chasmogamous flowers, but, since she does not observe the division of the generative cell, this cannot be regarded as conclusive.

Another method of behaviour is described in a recent description of the sperms of *Vallisneria* (22). Wylie shows how the division of the generative cell gives rise to two male cells. He describes the passage of these male cells down the pollen-tube and into the embryo sac, until they flatten themselves against the egg, still retaining their individuality. In some cases where more than one pollen-tube enters the embryo sac, all the sperms are clearly complete cells after they emerge from the tip of the pollen-tubes. Finn, in a recent paper on *Asclepias Cornuti*, describes male cells which retain their individuality after their penetration into the embryo sac, although he does not observe them in actual contact with the egg. Ishikawa (7) describes the male nuclei as being surrounded by a protoplasmic sheath while in contact with the egg, although he does not use the term 'male cell'.

In the rest of the more recent work the generative nucleus is described as giving rise to two male cells which retain their individuality while in the pollen-tube, but which are seen in the embryo sac as free nuclei, having shed their protoplasmic sheath either just before or just after their discharge from the pollen-tube. This third mode of behaviour of the male gametes is described by Welsford for *L. auratum* (20). In this case the protoplasmic sheath is shed just before their discharge from the pollen-tube.

This third way is the method of behaviour of the male gametes in *Viola odorata.*

Fertilization of the Egg.

About the time of entry of the pollen-tube into the embryo sac, the nuclear membrane of the egg appears to become less defined, and the nucleolus moves towards one side of the nucleus and seems to be pressed against the cytoplasm (Pl. XII, Figs. 3, 4, 5). In the seven cases of fertilization found, the nucleolus had in every one taken up a position on the side of the nucleus away from the pollen-tube (Pl. XII, Fig. 3). The male nucleus appears to move through the egg cytoplasm until it approaches the egg nucleus on the side against which the nucleolus is pressed. This means that its path to the egg-nucleus is an indirect one. Pl. XII, Fig. 3, shows it approaching the egg-nucleolus, in Pl. XII, Fig. 4, it is shown almost in contact. During this time the male nucleus may become rather irregular in outline while its nucleolus increases in size, and the reticulum passes into a spireme which does not seem to be continuous. Finally the male nucleus presses against the egg nucleus so that it appears to be in contact with the egg nucleolus (Pl. XII, Fig. 3). Apparently the two nucleoli fuse, since a stage has been found in which the nucleolus of the male has disappeared and only a darkly staining thread, pressed against the egg nucleolus, remains to represent the male nucleus (Pl. XII, Fig. 5). This preparation suggests that the spireme of the male gradually loosens and spreads through the nuclear cavity of the egg. Another preparation, thought to be a slightly later stage, shows part of the male spireme faintly outlined against the egg nucleolus. This suggests that as the male spireme loosens, it undergoes changes rendering it unstainable.

Finally no trace of the male nucleus can be seen, since the spireme is rendered completely unstainable, and the nucleus of the fertilized egg exists for a time as a resting nucleus in which almost all the stainable material is once again concentrated in the nucleolus, now centrally placed. Occasional scattered granules suggest a trace of a reticulum. By loss of the vacuole, the protoplasm of the egg becomes spherical instead of cup-shaped and a definite membrane is formed.

Although masses of deeply staining globules are discharged from the pollen-tube along with the male nuclei, none of these enter the egg cytoplasm but remain massed against it (Pl. XII, Figs. 3, 4, 6). Often they still remain when the fertilized egg begins to divide. It would seem that the male nucleus does not penetrate into the nuclear area of the egg while still retaining its individuality, but that while it is pressed against the egg nucleus the membranes between the two nuclei break down and a gradual mingling of the chromatin content of the two nuclei occurs.

There are so many points of difference that it is difficult to bring these facts into line with recent work on angiosperms. In no other case described does the egg nucleus, at the time of fertilization, appear in a stained prepara-

tion to consist entirely of a large nucleolus surrounded by a clear zone which has rather ill-defined limits. In *L. auratum* (Blackman and Welsford (1) both egg nucleus and male nucleus possess a very definite reticulum with a few small scattered nucleoli and a well-marked boundary, so that fusion occurs by the breaking down of the membrane between the two nuclei, even though the reticulum, which by this time has developed into a definite spireme, remains distinct during the early stages of the following division. Miss Bliss (2) in her work on the chasmogamous flowers of *Viola* certainly figures the egg nucleus at the time of fertilization with a large nucleolus and only a faint reticulum, but she does not stress this, while the figures suggest a very definite membrane. Unfortunately many investigators give no exact details of the process of fusion, only showing the two nuclei lying in contact with each other, while others figure the male nucleus with its membrane completely enclosed within the female nucleus. Many instances are recorded in which either both nuclei, or only the male, enter the spireme stage before fusion occurs, such is the case in *L. longiflora* (Weniger (21)), *L. grandiflora* and *L. martagon* (Nothnagel (14)). In all these cases the spireme is continuous and division continues on the breaking down of the membrane between the two nuclei, while the spiremes remain distinct and two groups of chromosomes form which are finally brought together on the spindle. In the present investigation it has been observed that the reticulum of the male may begin to pass into a spireme, but after fusion with the egg nucleus this does not segment into chromosomes, but the thread is lost sight of inside the egg membrane and a resting stage intervenes before the first division of the embryo. This condition has not been described in records so far published.

Triple Fusion.

As in the case of the egg nucleus at the time of fertilization, so by the time triple fusion occurs, no very distinct membrane can be seen round each of the polar nuclei, although there is hardly any suggestion of a fusion of the two (Pl. XII, Fig. 4). The second male nucleus travels towards them and becomes associated with one of them. At first it remains just outside this polar nucleus and undergoes certain changes (Pl. XII, Fig. 2). It does not remain in an ovoid form for long, but becomes spherical; the reticulum meanwhile becomes more deeply staining and beaded while the nucleolus grows in size (Pl. XII, Figs. 2 and 3). The reticulum now begins to pass into the spireme condition, the nuclear membrane begins to disappear and the nucleolus passes to one side (Pl. XII, Fig. 7, C_1 and C_2). It is at about this stage that the male nucleus makes its way into the nuclear cavity of the polar with which it is associated. In Pl. XII, Figs. 7, C_1 and C_2, the outline of the nuclear cavity of the polar is shown. Meanwhile the reticulum assumes the spireme condition, and the nuclear membrane of the male

entirely disappears, so that fusion of the two nuclei has occurred although the male nucleolus and spireme are still distinguishable. The nucleolus continues to grow in size while the spireme becomes more diffuse and now surrounds the male nucleolus. In Pl. XII, Fig. 7, all the changes which the male nucleus undergoes are shown; in 7, A it is figured as a male cell while still in the pollen-tube; in 7, B it is shown with its rounded outline and reticulate structure while just outside the polar nucleus; in 7, C_1 it is passing inside the membrane; while in 7, C_2 and C_3 it is completely inside and its reticulum has assumed a definite spireme. The male nucleolus approaches the polar nucleolus and grows still larger, while the male spireme spreads round the polar nucleolus until a stage may be reached when the three nucleoli (that is the male and the two polar nucleoli) are practically all the same size; in Pl. XII, Fig. 9, the beginning of this is seen. Even at this stage the two polar nuclei may be quite distinct from one another. As the three nucleoli become more equal in size due to the enlargement of the male nucleus, the spireme, after a certain point, becomes less definite and is broken up (Pl. XII, Fig. 9).

Finally the male nucleolus fuses with the polar nucleolus with which it is in contact. The spireme undergoes some change so that it can no longer be demonstrated by staining. For a short period, while the fusion nucleus rests before the first division of the endosperm, all the stainable material is concentrated in two nucleoli, which may still be partly separated by the original boundary between the two polar nuclei. One of these is usually larger and often ovoid in shape, and is presumably the one which has been formed from the fusion of one polar nucleolus and the male nucleolus, while the other is that of the second of the two original polar nuclei. These two nucleoli are seen, in de-stained slides, to be very vacuolate. Frequently the two nucleoli stain differently, so that while the larger stains deeply, the other may be nearly colourless. This fact is of special interest, since just before the fusion occurs the male nucleolus is often found to stain more deeply than the two polar nucleoli, both the latter may be almost colourless, while the male nucleolus is fully stained (Pl. XII, Fig. 8).

In spite of the difference in behaviour of the male nuclei, several interesting points of comparison can be made between triple fusion and the fertilization of the egg. The polar nuclei are very similar to the egg nucleus in that practically all their stainable material is concentrated in the nucleoli, both of which are surrounded by a clear zone which has no well-defined limits. In triple fusion the male nucleus develops a more definite spireme than was found in the other male nucleus, but in both cases the threads are rendered unstainable after fusion, and instead of the division proceeding, as one would expect from a comparison with other forms, both the fertilized egg and the primary endosperm nucleus remain in a resting condition for

a short period before the first division. Hence in triple fusion, as well as in the fertilization of the egg, it is difficult to bring the facts observed into line with other work on the subject. In every detailed account of triple fusion published the polar nuclei have a well-marked membrane and a very definite reticulum with one or more small nucleoli within it. In *L. auratum*, when the male and two polar nuclei become associated together, fusion occurs by the breaking-down of the membranes between them, while the spiremes (for usually the nuclei begin to divide before fusion) remain distinct until chromosome formation, so that the fusion nucleus does not pass into a resting condition before the first division.

That the male nucleus grows in size before fusion occurs has frequently been reported, but in this case growth is different. Usually the male nucleus increases in size while still bounded by the nuclear membrane, and still preserving its reticulum. In this case it is the nucleolus which increases in size both before and after the nuclear membrane has disappeared and while the reticulum is passing into a spireme (Pl. XII, Fig. 7).

The fact that the male fuses with one of the polars first is not unusual, the same thing has been reported by Welsford (20) for *L. martagon*, where the male nucleus first fuses with the upper polar nucleus and for *Monotropa uniflora* by Shibata (19). Usually, however, the second polar nucleus soon fuses with them and the membranes between the nuclei completely break down. It is apparently unusual for one polar nucleus and the fusion nucleus of the male and other polar nucleus to remain distinct even after the nuclei have passed into a resting condition.

Endosperm Formation.

The primary endosperm nucleus begins to divide while the fertilized egg is still resting. A stainable thread appears which is at first very faint and beaded but which afterwards thickens. At this stage all trace of the original boundary between the two polar nuclei is finally lost, and for a short time before, and during, the breaking-up of the thread the endosperm nucleus is ovoid in form with a very definite nuclear membrane, and with two nucleoli one of which is usually larger than the other (Pl. XII, Fig. 10). During this division, also, the cytoplasm round the dividing nucleus tends to round itself off and become separate from the remaining cytoplasm in the embryo sac, with which it was previously connected (Pl. XII, Figs. 10 and 11). The spireme is never continuous and is usually massed in two portions round the two nucleoli (Pl. XII, Fig. 10). The thread breaks up into thirty chromosomes, which become very much scattered, while the nuclear membrane disappears, and before the chromosomes become arranged on the spindle they are often spread over quite a large area of protoplasm, while the two nucleoli are often far apart.

When the spindle is formed the chromosomes, in the few cases observed,

are seen to collect in two groups side by side. In one the chromosomes are seen in equatorial view, while in the other they are seen edgewise (Pl. XII, Fig. 11). The diploid number of chromosomes (20) have been counted in one group, and the haploid number (10) in the other. The two groups of chromosomes remain distinct, since at telophase two masses of rather indistinct chromosomes may be seen side by side at either end. More stages of this division are needed to interpret exactly the behaviour of the two groups of chromosomes during metaphase and telophase. At telophase the chromosomes are pulled into a very tight mass which afterwards loosens. As the two daughter nuclei re-form, the two separate groups in each become associated. The daughter nuclei are very much elongated, and possess either one large ovoid nucleolus and one smaller spherical one, or three spherical nucleoli of equal size, as well as other globules of stainable material (Pl. XII, Fig. 12). Suggestions of the two groups of chromosomes are seen for the first few divisions of the endosperm, and in the same way for the first three or four divisions the resting nuclei possess either one large ovoid nucleolus and one smaller spherical one, or three spherical ones of equal size.

These facts differ from those given in other records chiefly in that spireme and the chromosomes are divided into *two* groups instead of *three* as one would expect; while the two groups of chromosomes appear to remain separate right up to the telophase of the division, instead of associating soon after metaphase. Nothnagel (14), in a paper on 'Fecundation and Formation of Primary Endosperm Nucleus in certain Liliaceae', describes how in *Trillium grandiflorum* spiremes of the male and two polar nuclei remain separate even when the three nuclei have fused, and three groups of chromosomes are formed which are at first scattered on the tripolar spindle. The spindle becomes bipolar, and all the chromosomes are then drawn on to the equatorial plate, the groups remaining separate for a short time but not being distinguishable after this stage.

The facts obtained from the present investigation seem to suggest that in *V. odorata* the fusion between the male and the first polar nucleus must be a very complete one, since the observations of the earlier stages of triple fusion point to the fact that the diploid group of chromosomes and ovoid nucleolus represent the chromatin content of the male and one polar, rather than of both polars, which might have been assumed had only the last stages of fusion been found.

The First Division of the Embryo.

The fertilized egg does not begin to divide until four endosperm nuclei have been formed, so that it rests for a much longer period than does the primary endosperm nucleus. No detailed work has been done on this division, but it appears to be perfectly regular and suggests that a complete

fusion has occurred. The diploid number of chromosomes (20) can be counted at the metaphase of the first division, and no suggestion of a grouping corresponding to that found in the first division of the endosperm has been observed.

Theoretical Considerations.

Two minor points of interest have arisen out of the present investigation. They are concerned with the number of chromosomes, and the germination of the different forms of pollen-grain.

Chromosome Number.

The haploid number of chromosomes has been shown to be 10. This is an unusual chromosome number, and other records of chromosome counts for *Viola* were examined to see if this were a feature of the order. Although no mention of *V. adorata* is made, Ishikawa (8) in his list of the number of chromosomes, counted in Violaceae by Miyaki (12), mentions nine *Viola* species. It is of interest that in three of these, *V. grypoceras*, *V. verecunda*, and *V. nipponica* the haploid number of chromosomes recorded is 10. For the other species the numbers are 6, 12, 24, and 36, all multiples of two and three, and therefore more usual numbers.

Germination of the Different Forms of Pollen-Grain.

The germination of some pollen within the anthers of the chasmogamous flower has been recorded elsewhere. Leclerc du Sablon (10) and Goebel (4, 5) note that it occurs in the chasmogamous flowers of *V. sylvatica* and *V. biflora*. It does however seem unusual to find it to the extent described for *V. odorata*, var. *praecox*, where a tangled mass of germinating pollen-grains may be found inside the undehisced anthers, and seeds are never set. No mention, however, has been found of any variation in the shape of the pollen-grains which germinate in the two forms of flower, as is described in the present investigation. It will be remembered that while the large ovoid grains and the smaller ridged grains occur in the anthers of both chasmogamous and cleistogamous flowers of *V. odorata*, var. *praecox*, it is the ovoid grains which germinate freely in the chasmogamous flowers and the round ridged grains which function in the cleistogamous flowers, and it was suggested that the ovoid pollen-grains pass through a round ridged form in the course of their development.

It is conceivable therefore, if this assumption be true, that the germination of the pollen-grains in the cleistogamous flower while yet in their round ridged form is an indication of the arrested development which Goebel (4) describes. He finds that a comparison of the development of chasmogamous and cleistogamous flowers shows that the latter are arrested in development,

although the sexual organs become mature. It is suggested that in the cleistogamous flower of *V. odorata*, var. *praecox*, the pollen-grains mature at an earlier stage of development than they do in the chasmogamous flower. In the chasmogamous flower the round ridged pollen-grains always assume an ovoid shape before germinating, while in the cleistogamous flower, if they do not germinate while at the round ridged stage, they then become ovoid and degenerate.

Following on this assumption it would seem that the germination of the pollen-grains within the anther in the chasmogamous flower may have been the first stage towards the initiation of cleistogamy, rather than a secondary feature which has arisen later as a result of it. Goebel is hinting at the same thing when he says 'I should like to regard the capacity of the pollen-grains of sending out tubes within the anthers without the attraction of the stigmas, not as the last step in the transformation, but as the first basis of hypothesis for the possibility of cleistogamous flower formation'.

SUMMARY.

1. *Viola odorata*, var. *praecox*, G. bears flowers throughout the whole year. These are of three types, chasmogamous, semi-cleistogamous, and cleistogamous. Seeds are set only by the last two types.

2. The semi-cleistogamous flowers show every transition between chasmogamous and cleistogamous flowers as regards (*a*) size of flower, (*b*) bending and shortening of the style; but they resemble cleistogamous flowers in (*c*) the complete absence of spur and nectary, and (*d*) the type of pollen-grain that germinates.

3. Pollen-grains of at least two forms are to be found in the anthers of all three types of flower. These are described as (1) ovoid or tetrahedral grains, and (2) round ridged grains. There is reason to believe that in the chasmogamous flower the round ridged grains are merely the immature ovoid grain, but that in the cleistogamous flower they are mature in the round ridged condition. If by any chance they should develop into the ovoid form, they degenerate.

4. Germination of the pollen-grains in great profusion inside the undehisced anthers is a marked feature of all three types of flower. In the chasmogamous flower it is the ovoid pollen-grains which germinate and most of the pollen-tubes remain inside the anther. In the cleistogamous and semi-cleistogamous flowers the round ridged pollen-grains germinate and the pollen-tubes grow through the anther wall and out on to the stigma, while the ovoid grains degenerate.

5. The existence of definite male *cells* (not merely nuclei) in the pollen-tubes of both chasmogamous and cleistogamous flowers has been

demonstrated. These are pear-shaped when first formed, but later become ovoid.

6. At the time of fertilization the egg and polar nuclei have all their stainable material concentrated in a large nucleolus. The egg-cell is pressed against one of the synergids so that the protoplasm of the two is almost continuous.

7. The pollen-tube passes through the other synergid and discharges its contents on to the egg-cell. At this stage it possesses numerous globules of deeply stainable material which surround the egg but do not penetrate its protoplasm. The protoplasmic sheath of the male cells is shed about this time, and they are found in the embryo sac as free ovoid nuclei possessing a definite reticulum.

8. True fertilization has been shown to occur. The reticulum of the male nucleus passes into a spireme during fusion with the female nucleus, but the fertilized egg passes through a resting condition before the first division takes place.

9. During triple fusion the other male nucleus fuses completely with one polar nucleus. Its nucleolus increases in size, and its reticulum forms into a spireme as the male approaches the polar nucleus. Its membrane breaks down, and it makes its way into the nuclear cavity of the polar, but the nucleolus and spireme are distinct for some time. Finally, however, the two nucleoli fuse. Meanwhile the other polar nucleus, although associated with the fusion nucleus, may remain distinct during the resting period that ensues, after the spireme is rendered unstainable, until the first division occurs.

10. In the first division of the primary endosperm nucleus two groups of chromosomes are seen, one containing the diploid, the other the haploid number of chromosomes. These may still be distinct in early telophase, while in the resting condition of the daughter-nuclei resulting from this and the next two divisions, either one large ovoid nucleolus and one small one, or three smaller spherical nucleoli, are always found, suggesting that the fusion of the chromatin contents of the one polar nuclei and the fusion nucleus formed by the union of the male nucleus and the other nucleus, is still not complete.

11. The haploid number of chromosomes is found to be ten.

This work was carried out in the Botanical Laboratories of the Royal Holloway College, and I should like to thank Miss Blackwell for the help and inspiration I received.

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DESCRIPTION OF PLATE XII.

Illustrating Dr. Margaret A. P. Madge's paper on Spermatogenesis and Fertilization in the Cleistogamous Flower of *Viola odorata*.

All the figures were drawn with a camera lucida under a Zeiss 2 mm. oil-immersion lens. N.A. 1·32. Figures 2–6 and 12 were drawn under a Zeiss Ocular 12, and figures 1 and 7–11 under a Zeiss Ocular 18.

The following abbreviations are used for the names of fixatives and stains.

Fixatives: Flemming's weak solution Fw. Allen's modification of Bouin's Fluid AB.
Stains: Flemming's triple Ft. Heidenhain's haematoxylin Hh.

Figures 1–6. *Fertilization in the Egg of the Cleistogamous Flower.*

Fig. 1. Pollen-tube in the stylar canal with two oval male cells showing elongation of the male nuclei, and a tube nucleus which is breaking up into globules of chromatin. AB. Hh.

Fig. 2. An obliquely longitudinal section through the two synergids, egg and two polars, soon after the discharge of the pollen-tube. The two male nuclei can be distinguished, one near the egg nucleus, the other near the polar nuclei. The nuclear cavity of both egg nucleus and polar nuclei

can be recognized; the nucleoli are found in the next section. Some of the darkly staining globules discharged from the pollen-tube with the two male nuclei, are seen. AB. Hh.

Fig. 3. A transverse section of the two synergids and egg, showing one male nucleus, possessing a spireme, approaching the egg nucleus. The egg nucleolus is found to one side of the egg nucleus, and darkly staining globules from the pollen-tube can be seen. AB. Hh.

Fig. 4. A longitudinal section of synergids, egg, and polars, showing one male nucleus near the egg nucleolus. The two polar nuclei are seen below the egg. The second male nucleus is not shown. AB. Hh.

Fig. 5. A transverse section through the synergids and egg showing the darkly stained spireme of the male nucleus pressed against the egg nucleolus. Masses of darkly stained material surround the egg. AB. Hh.

Fig. 6. A slightly later stage than the one shown in Fig. 5, the male spireme has almost disappeared. AB. Hh.

Figures 7–9. *Triple Fusion.*

Fig. 7. Showing the changes the male nucleus passes through during triple fusion.

(1) Male cell in the pollen-tube, showing the elongated form of the male nucleus AB. Hh.

(2) Male nucleus as it approaches the polar nuclei, more rounded in form, with a deeply staining reticulum. AB. Hh.

(3)-(5) After the male nucleus has passed into the nuclear cavity of one of the polar nuclei, showing the gradual increase in size of the male nucleolus and the formation of a well defined spireme. AB. Hh.

Fig. 8. (1) and (2). Two sections through the polar nuclei in the same embryo sac after the male nucleus has passed inside the nuclear cavity of one of them. The male nucleolus is much more deeply stained than the nucleoli of the two polars. AB. Hh.

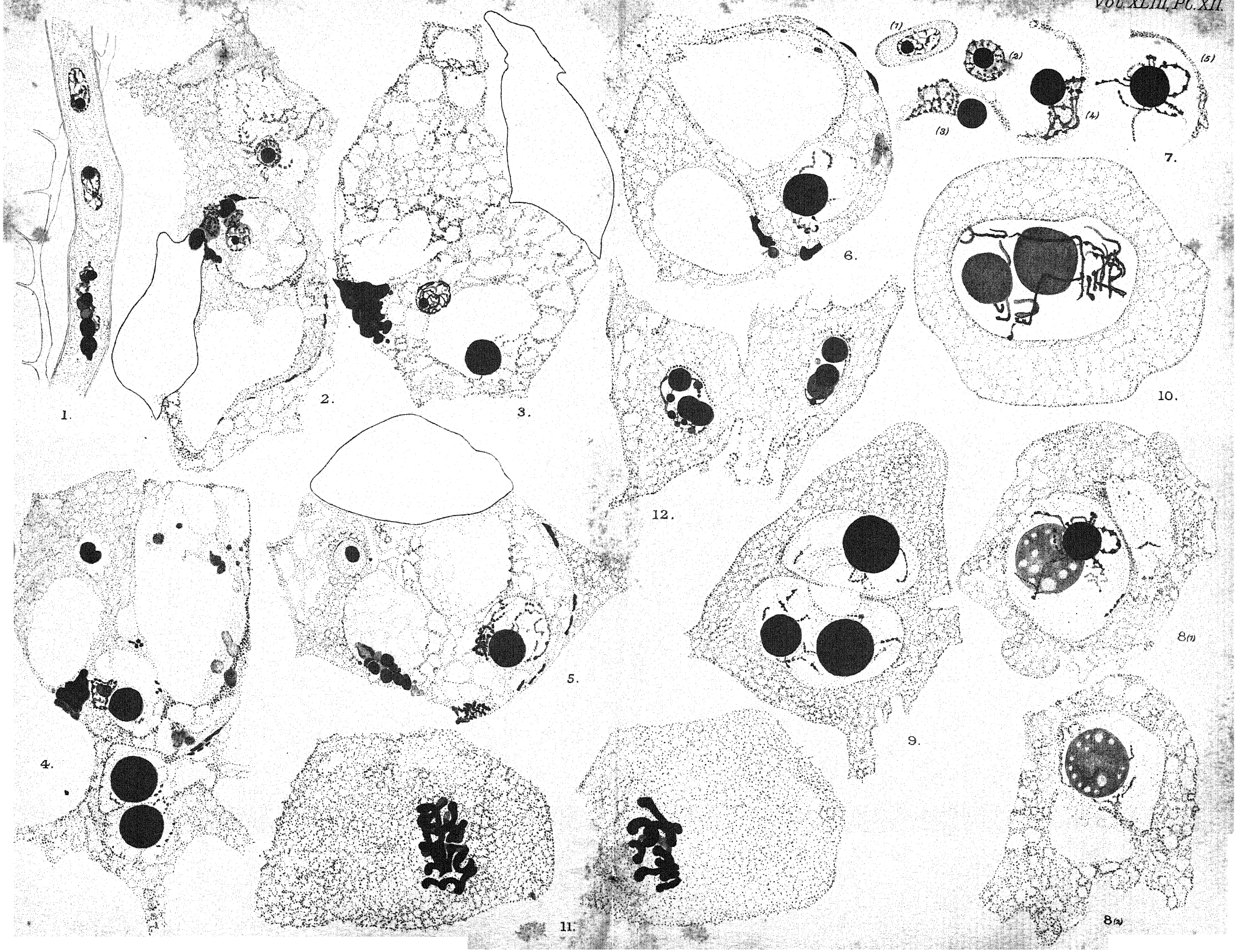
Fig. 9. A later stage showing the further increase in size of the male nucleolus while inside the nuclear cavity of one of the polars. The spireme has almost disappeared. AB. Hh.

Figures 10–12. *Division of the Primary Endosperm Nucleus.*

Fig. 10. Thickening of the spireme and formation of a definite membrane round the primary endosperm nucleus. The protoplasm round the nucleus is delimited by a thin membrane. AB. Hh.

Fig. 11. A and B. Two successive sections showing the formation of two groups of chromosomes, one with the haploid, the other with the diploid number. AB. Hh.

Fig. 12. The two daughter nuclei formed from the division of the primary endosperm nucleus. Each contains three spherical nucleoli. AB. Hh.


1.
2.
3.
4.
5.
6.
(1)
(2)
(3)
(4)
(5)
7.
10.
12.
9.
8(1)
8(2)
11.

Saltations in Bacteria.[1]

II. Bacillus prodigiosus.

BY

J. C. RAMCHANDANI.

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With Plate XIII.

THE work of Nirula (14) opened up a new line of investigation into the phenomena of microbic dissociation which seems already to have given results of some considerable interest and which gives promise that further research on the same lines may do something to advance our knowledge of the course of evolution.

At the suggestion of Professor Paine *Bacillus prodigiosus* was selected, since, being strongly pigmented, any differentiation on prolonged cultivation, as practised by Nirula, would probably be reflected in colour in the progeny of the differentiated cells. In this way sectors arising by saltation might possess less or more colour than the parent, and any new strain produced would have its stability or otherwise portrayed in easily recognizable colour phenomena.

It is a common experience that when *Bacillus prodigiosus* is cultivated at blood heat it loses its power to produce the normal red colour but that the non-pigmented strain reverts at once to the pigmented condition when sub-cultures from it are incubated at lower temperatures. Wolf (17) working with various pigmented bacteria observed that certain individual colonies varied considerably in the amount of colour they possessed, some being paler and others darker than the majority. By selecting these abnormal colonies for further cultivation he expected that the colour differentiation which had arisen spontaneously would be established as a permanent characteristic in the various strains obtained. This, however, was not realized. He subsequently sought to induce mutations by chemical and physical means, and here some success attended his efforts for he was able to produce one white and four dark races, the white race arose after

[1] Saltations in Bacteria, by R. L. Nirula, which appeared in this journal, vol. xlii, pp. 431–66, 1928, forms Part I of this series.

fourteen transplantations on solid media containing a considerable dose of mercuric chloride, the cultures being incubated at 37·5° C. The dark red strains were obtained by similar cultivation in media containing small quantities of either potassium bichromate, cadmium nitrate, or potassium permanganate. No reversion of these strains occurred when grown subsequently on ordinary media at 37·5° C. This author states that a dark race was obtained by the use of potassium bichromate even at room temperature. Baerthlein (2) in a short note described certain variations from the normal type which appeared on plating from old cultures of *Bacillus prodigiosus*. Thus he obtained dark red, pink, white, white colonies with red spots, and red colonies with whitish sectors. The colonies did not differ from the original solely in colour, but many showed also differences in texture, being of a more slimy consistency. Further, there existed certain morphological differences between the individual cells of red and white colonies. The characteristics of the various strains were retained when these were cultivated on different media and even after *passage* through animals. Baerthlein found that red strains incubated at 37° C. immediately lost their power to produce pigment, but that they regained their colour when cultivated at lower temperatures. Migula (13) points out that permanent white races can be obtained by continually growing the red ones at high temperature. Finally, Enderlein (7) observed the occurrence of brilliant red secondary colonies on primary white colonies of *Bacillus prodigiosus* growing on nutrient agar containing from 6·5 to 8 per cent. of sodium chloride.

Experimental.

The strain of *Bacillus prodigiosus* employed in the experiments about to be described was obtained from the collection of Type Cultures of the Lister Institute, and had been maintained as a stock culture in this laboratory for some years. In the commencement several single cell cultures were obtained by the method described by Paine and the author (16), a modification of the method of Paine (15). Transfers from these cultures were made by point inoculation of the surface of a layer of nutrient agar five or six millimetres deep, one inoculation being made in each quadrant of the dish so that colonies, separated from each other by some five centimetres, should have freedom of growth uninfluenced by diffusion of inhibitory substances from neighbouring colonies. In the first experiment cultures were made on ordinary bouillon agar, but later experience showed that the organism was more prone to exhibit phenomenon of saltation on the synthetic medium of Brown (4) designed for the culture of *Fusarium*. One hundred plates were used in this first experiment. After inoculation from the single cell culture the plates were incubated at a temperature of 24° C. The first appearance of sectors was noticed four days after the setting up of the experiment.

These sectors occurred in about 5 per cent. of the four hundred colonies, and were observable at the margin as small wedge-shaped areas of a paler colour than that of the parent colony. On prolonged incubation during a further twelve days the colonies increased in size to some fifteen millimetres diameter and the sectors developed into striking features in the colonies, so that on the sixteenth day more colonies were found to have produced sectors, and those which had previously shown sectors were now found to have produced many more. In some cases this had occurred to such a degree that the colony was almost completely surrounded by them. This multiple development of sectors in certain colonies formed so striking a contrast with the total absence of sectors in the majority of the colonies as to suggest that saltation arises in the progeny of cells already differentiated from the majority by the possession of a tendency in this direction. This question has not been looked into further in the present research, but it seems to possess so much fundamental importance as to warrant its close investigation at a later time.

In the early stages of experimentation the sectors possessed a rather pinkish white colour, and in certain cases a marked streaking of the sector with red rays was noticeable (Pl. XIII, Fig. 1). On culturing from these early sectors all colonies developed with the colour of the parent. The mode of inoculation for the preparation of these subcultures was the placing of a number of points from the red portion, or parent part of the colony, in the same dish with a number of points from the sector, or saltant part, and although, at first, no difference could be observed in the colonies representative of these two groups yet, in every experiment made, it later became noticeable that far more sectors developed on the colonies of the saltant than on those of the parent. The impression was gained that these pink sectors represented the growth of more than one original cell, the saltant being mixed with red cells in varying proportions. Mostly the mixture of cells was so intimate as to cause uniformity of colour throughout the sector, but, occasionally, as in the colony of (Pl. XIII, Fig. 1) the red cells, being perhaps considerably in the majority, became grouped in certain regions and their progeny provided the streaks of deeper red. Attempts were made to obtain the pure line strain of the saltant by single cell culture from the apparently mixed population of the sector. In these attempts the red parent type seemed more easily obtainable than the saltant, and before success attended the efforts in this direction fortune favoured the author and made further attempts unnecessary; there appeared on one of the colonies of the parent a clear-cut white sector of absolute purity (Pl. XIII, Fig. 3). In transferring from this colony the colonies representative of the sector assumed a pure white (Pl. XIII, Figs. 2, 3A, and 4), and since its formation to the present time, about nine months, during which time it has been subcultured over thirty times, the strain has remained perfectly white. It has,

however, undergone further saltation and given rise to a sector of a more transparent type, although still without colour.

One had then the original strain of *Bacillus prodigiosus*, O; the first saltant white strain, I; and the second saltant white strain IA (Pl. XIII, Fig. 4). These three strains were compared most carefully by culture on various media to determine if any differences existed in their morphological, cultural and physiological characteristics. Apart from the above-mentioned colony characters of colour and transparency no differences whatever could be found, not even such differences in rate of growth and speed of reaction as were observed by Nirula (14) in the strains of organisms with which he was concerned. There did, however, appear certain points of divergence from the published description of the behaviour of *Bacillus prodigiosus*, Chester (5), Mehta (12), Bergey (3). These describe a motile organism giving gas by fermentation of glucose and sucrose, and besides Mehta found no acid from lactose. The original strain which forms the subject of this paper was one of intense red colour, becoming purple in old cultures on certain media. The individual cell was almost a coccus, and was never observed to possess motility. The absence of motility in certain strains of *Bacillus prodigiosus* has previously been noted by Lehman and Neumann (11). It produced acid, but no gas, in media containing glucose, levulose, sucrose, maltose, lactose, raffinose, mannitol, salicin and inulin, but no acid in media containing dulcitol and dextrin.

By the courtesy of the staff of the Lister Institute in charge of the National Collection of Type Cultures it has been possible to investigate the serological reactions of these three strains. Immune sera were obtained from each strain, the titre of the original being $\frac{1}{1000}$, that of Strain I being $\frac{1}{4000}$, and that of Strain IA being $\frac{1}{5000}$. With each of these sera the three strains reacted in precisely the same manner.

The two white strains have been inoculated into mice with the following result. Those infected with Strain O and I died, but the mouse inoculated with strain IA, although the dosage was a considerable one, remained alive, a loss of pathogenicity which forms, perhaps, the most striking change so far exhibited by any of these saltant strains investigated in this laboratory. The heart-blood of the two dead animals yielded cultures of the red and white *Bacillus prodigiosus* unchanged through this one animal *passage*.

Attempts to induce Reversion in the New White Strains.

Various methods of culture have been employed with the object of producing, if possible, a return to the pigmented condition in the new strains which have arisen by saltation.

In the first series the effect of temperature was investigated. Four different media were inoculated each for three colonies of the parent, three

colonies of Strain I, and three colonies of Strain IA. The special media used were bouillon agar, Brown's synthetic agar (4), potato extract agar and prune juice agar. Five plates of each were incubated at temperatures of 10°, 20°, 30° and 37° C., observations and measurements of the colonies were made at intervals of three days up to fifteen days, after which no further growth took place. The measurements showed no differences in rate of growth between the three strains, the rate and development being relatively low at both 10° C. and 37° C. as compared with the rate and development at the intermediate temperatures. No colour returned to the white strains, but there was a complete suppression of colour in the parent strain at 10° and 37° C. This suppression of colour was merely a thermal inhibition, for, as has been frequently observed by others for the higher temperature, the colour returned at once to the organisms which developed on further incubation at temperatures between these extremes.[1] By alternately incubating a culture at high and medium temperatures, allowing three days at 37° C. where the growth rate is low, and two days at 24° C. where the growth rate is high, the colonies of the parent organism developed in alternating white and red zones giving the target effect seen in Pl. XIII, Fig. 5, the white zones assumed a faintly pink colour on exposure to the lower temperature, so faint a colour as to suggest that its presence was due to a small percentage of young cells still possessing the power to produce colour, the older ones being assumed to have the chromogenic mechanism either destroyed or in abeyance.

In the second series the effect of alteration of the concentration of hydrogen-ion was determined. A considerable number of plates in a range of pH 5·0, pH 7·1 and pH 9·0 were inoculated with the three strains and incubated at 24° C. No change in the pigmentation of the colonies was observed, and there was no differentiation of the strains in their rate of development, multiplication of the organisms was however greatest at the alkaline end of the range. Attempts were made to induce reversion by cultivation in media containing small quantities of alcohol. Development occurred in concentrations from one to five per cent. with gradually diminishing rate and a lessening tendency for pigment formation in the parent strain. Pl. XIII, Fig. 6, shows the effect of one per cent. and five per cent. alcohol respectively. At five per cent. the colonies were only faintly pink, but transfers to alcohol-free media produced an immediate return of the parent to its normal pigmented condition. No effect showed in colonies of Strains I and IA.

[1] As mentioned earlier both Migula (13) and Wolf (17) have stated that under certain conditions red strains of *Bacillus prodigiosus* yield permanent white strains if subjected to prolonged incubation at 37° C. An experiment with the present organism was continued for three months. During this time cultures were incubated at 37° C., transfers being made at approximately weekly intervals. At each transfer period duplicate cultures were carried to lower incubation and, throughout the course of the experiment, transfers incubated at 24° C. have immediately assumed the deep rich colour of the parent unimpaired.

Not possessing any data upon which ideas as to the nature of the colour-forming mechanism could be formulated, acting on a suggestion that it might be temporarily suppressed in the saltant by the loss of some essential part, as for instance an activator, the author was led to attempt to influence the organisms of a developing colony of the white saltant strain by some thermostable soluble product of the red parent. To this end dead cells of a growth of *Bacillus prodigiosus* heated at 54° C. were deposited in the neighbourhood of the developing colony of the colourless strain. Apart from a restriction of growth causing a flattening of the margin of the colony no effect was observable. Similarly no effect was shown by a colony of the white strain purposely planted near to a colony of the red parent.

At this stage access was obtained to the paper of Wolf (17) previously cited. The toxic substances employed by him in procuring permanently altered strains of *Bacillus prodigiosus* were added to media, repeating, as nearly as possible, the proportions and conditions as given in his description. The results of these experiments showed complete variance from the results recorded by Wolf; where he obtained dark red strains with mercuric chloride, potassium bichromate and cadmium nitrate the effect produced on this organism was quite the reverse; at 37° C. only complete loss of colour in the parent strain was observed, no effect whatever being produced on the white strains. At the lower temperatures of 20° C. employed by Wolf, at which when grown in presence of potassium bichromate, his organism produced a relatively darker strain, no difference of colour on treated media and on untreated controls appeared in the colonies of either the parent or the white strains. Further, the author found complete inhibition of growth by the use of cadmium nitrate and an almost complete inhibition by the use of cadmium sulphate at 37° C. Only one conclusion can be reached: that Wolf's organism was an entirely different strain from that of the author. From the fact that Wolf found colour in his organism at the temperature of 37·5° C. it would appear that he was using an atypical *Bacillus prodigiosus*, if indeed his organism was correctly referred to this species.

During the course of the investigation old cultures have frequently exhibited the presence of secondary colonies (Pl. XIII, Fig. 7). Such secondary colonies were first observed in 1895 and described by Günther (9) and exhibited in photographs by Eisenberg (8). Enderlein (7) attributed the formation of red daughter colonies on primary white colonies of *Bacillus prodigiosus* to the presence of an excessive amount of salt in the medium. Enderlein's experiments have been repeated, and it has not been possible to confirm his results with the strain of *Bacillus prodigiosus* here employed. However, red secondaries have frequently appeared on white primary colonies when these have been transferred from 37° C. to air temperature; they have been shown to arise through the death of the majority of the white

cells the red secondaries being just normal developments from isolated cells still possessing the power of growth.

Summary.

1. Saltation has occurred in cultures of *Bacillus prodigiosus* resulting in the formation of a white sector in the colony.

2. The white strain has again shown saltation to yield a second white but more transparent strain.

3. The white strains have been shown to be unchanged from the original red strain morphologically, physiologically, and serologically.

4. Both white strains have remained true to colour for nine months during which they have been submitted to various chemical stimuli and also to *passage* through the mouse.

5. The second white strain has been shown to have lost pathogenicity for the mouse.

6. Red secondary colonies appearing on old primary colonies which have lost their colour through high incubation temperature have been shown to be normal red developments on transference to lower temperature of the few cells of the old colony which have retained their power of germination.

In conclusion the author desires to express his thanks to Dr. R. St. J. Brooks and his assistant Miss K. M. Rhodes of the Lister Institute for their kindness in making animal inoculations and preparing the immune sera; also to Professor S. G. Paine at whose suggestion the research was undertaken, for his constant interest and encouragement throughout the work; and to Mr. H. Tooley for his able assistance in taking the photographs.

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EXPLANATION OF PLATE XIII.

Illustrating Mr. J. C. Ramchandani's paper on Saltations in Bacteria II.

Fig. 1. Sector of impure saltant showing red rays of the parent strain.

Fig. 2. Plate with three colonies of the parent and six colonies of the saltant.

Fig. 3. Colony of *Bacillus prodigiosus* with sector of pure white saltant strain.

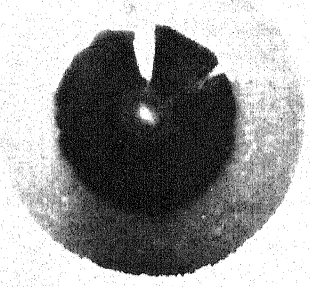
Fig. 3 A. Another example of saltation in the parent strain. The pure white colonies are of the saltant shown in Fig. 3.

Fig. 4. Plate with three colonies of O, the original strain of *Bacillus prodigiosus*; I, the first white strain; I A, the second white strain.

Fig. 5. Red and white zones as the result of alternating incubation at 37° C. and 24° C. of a colony of *Bacillus prodigiosus*.

Fig. 6. The effect of alcohol in reducing the colour of *Bacillus prodigiosus* A, three colonies on media containing 1 per cent. alcohol; B, three colonies on media containing 5 per cent. alcohol.

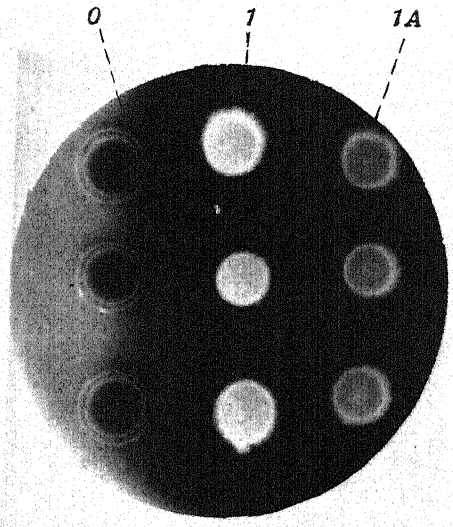
Fig. 7. Old colony after long incubation at 37° C. showing development of red secondary colonies when transferred to air temperature.



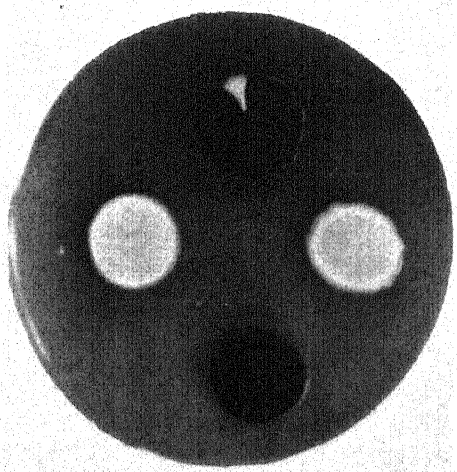
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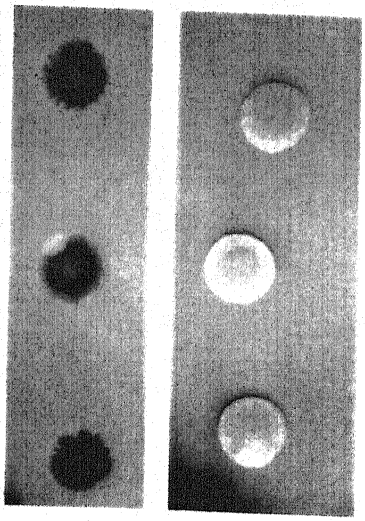
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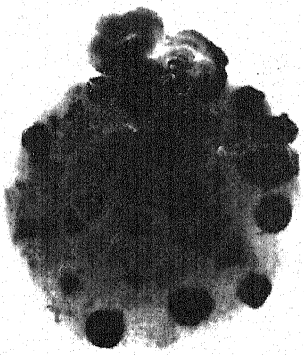
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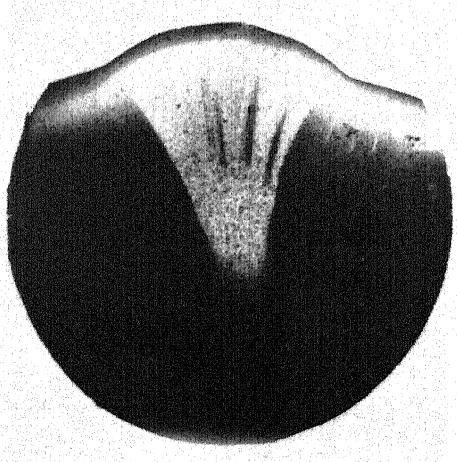
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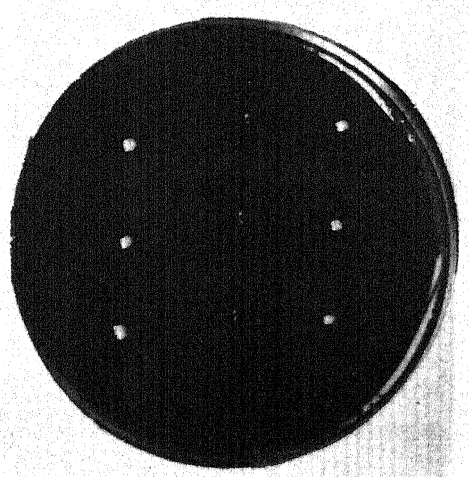
A **6** *B*



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Huth, London.

The Immediate Effect of Change of Light on the Rate of Photosynthesis.

BY

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(*Nankai University, Tsin-tien, China.*)

With six Figures in the Text.

I. INTRODUCTION.

IN the autumn of 1927 one of our students in Plant Physiology, who was experimenting on the effect of coloured light on photosynthesis by the bubble-counting method, noticed a very interesting phenomenon. When a coloured-glass filter was inserted between the source of light and the plant material (a shoot of *Elodea*), the latter immediately ceased to emit bubbles. After half or one minute bubbles first appeared slowly and then gradually increased until a constant rate was reached. When the filter was lifted, the number of bubbles emitted per unit time suddenly increased to a very high rate and then gradually dropped to a constant rate. It seems that the coloured light exerts an initial inhibiting effect, and the white light an initial accelerating effect on photosynthesis. This fact was immediately brought to the author's attention. Then we repeated this experiment with several other kinds of coloured-glass filter and with different *Elodea* shoots. The results confirmed the previous observation. This led us to several more problems, which are outlined as follows:

(1) Whether this initial inhibition-acceleration effect due to changed light occurs in other species?

(2) Whether this initial inhibition or acceleration is due to change in light intensity or due to change in colour; by colour filters both quality and intensity are changed?

(3) What is the probable explanation of this initial inhibition or acceleration.

In going over the literature on photosynthesis available to the author only one reference was found which mentioned the initial effect of light change on photosynthesis. Bose (4), in his monograph on the physiology of photosynthesis, maintained that when the plant is subject to increased

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intensity of light a certain period must elapse before photosynthesis attains a steady value under the increased intensity. He made only two experiments to substantiate his statement. In one he observed the effect, and in the other he failed to observe any. Spoehr (5) had also made a general statement in his recently published monograph. He stated 'that after changing any of the external conditions some time must elapse before a constant rate of bubble emission is attained'. As to the nature of the adjustment very little has been said.

In order to elucidate the points listed above it was felt that further work was necessary. Consequently in November of the same year the author began to work on the problem, and continued for two months. The work, however, had to stop on account of shortage of experimenting material. In the later part of April of 1928 this work was resumed and continued for two more months.

II. Method and Procedure.

(1) *The Plan of the Experiment.*

In this study three series of experiments were conducted, one following another. The total number of experiments in these three series was seventy-one. The first series of experiments started in 1927 and continued to 1928. In this series effort was concentrated on determining the initial effect of alternation of ordinary electric light and coloured or filtered light on photosynthesis. The plant material used in the 1927 experiments was *Myriophyllum*, cultured in our laboratory. Three species were used in the 1928 experiments. They were *Myriophyllum*, *Ceratophyllum*, and *Potamogeton*, which were collected directly from the pond on our campus before the experiments.

The second series of experiments concentrated on the study of the initial effect of the alternation of light of different intensity on photosynthesis, and the third on the study of the initial effect of the alternation of ordinary electric light and the coloured light with the same amount of available energy in the light. By available energy in light we mean that part of radiant energy which can be absorbed by chlorophyll and utilized in photosynthetic reaction.

(2) *Apparatus.*

The apparatus used in our experiments was very simple. The alternation of ordinary electric light and coloured light in our first series of experiments was obtained by the following simple device. The experimental material was placed in a test-tube which was filled with 0·02 mol. solution of $NaHCO_3$, and was placed in a beaker filled with water. The temperature of the water was kept constant at 23° C. The beaker was placed in the front

of the aperture of a tin box, in which a hundred-watt lamp was installed. The test-tube with the experimenting material was always placed 10 cm. from the centre of the bulb. On the aperture of the tin box a sliding device was provided, so as to facilitate the application and withdrawal of the coloured-glass filter. The alternation of light was produced by application and withdrawal of a coloured-glass filter. By changing different coloured-glass filters alternation of different coloured lights was obtained.

The Wallace M. and S. coloured-glass filters obtained from the Central Scientific Co., U.S.A., were used. The following were selected for our experimental use: (1) red 1, with transmission of 760–650 $\mu\mu$; (2) red 2, 760–610 $\mu\mu$; (3) red 3, 760–580 $\mu\mu$ and 510 to the end of violet; (4) orange, 760–540 $\mu\mu$; (5) green, 585–495 $\mu\mu$ (also a low luminosity band 740–710 in bright light); (6) blue, 520–350 $\mu\mu$ (also a low luminosity band 740–700 in bright light).

The light of different intensity was obtained by varying the distance between the light source and the plant material. In order to obtain an instantaneous change of light intensity a quick shifting of either plant material or light source was necessary. The shifting of the test-tube may cause great disturbance of the position of the plant, while the shifting of the light source was rather cumbersome. The present arrangement is to take two 100-watt lamps attached with a double switch. Thus one light will be out and the other on in a fraction of a second. The definite position of the lamp is marked on the experimental table. The two successive positions are so arranged that they are in an approximately straight line, and at the same time the front bulb will not obstruct the radiation from the farther bulb. The distance between the centre of the bulb and the plant material varies from 10–25 cm.

The alternation of lights with the same amount of available energy but of different colours is obtained by the following device. The lamp in the tin box with coloured-glass filter is attached to one end of a double switch, and another 100-watt lamp to the other end. The distance between the plant material and the filtered light is fixed by trial, and the final distance is that at which the plant will emit the same number of bubbles per unit time under both lights. When the distance is set, by changing the connexion of the switch we obtain the desired alternation. In this arrangement we assume that the same amount of available energy brings forth equal photosynthetic activity in the same plant.

(3) *Method of Measuring Photosynthetic Activity.*

In our work the bubble-counting method is employed in measuring the photosynthetic activity. The advantages and disadvantages of this method have been discussed at length by Spoehr (5). He finally comes to a conclusion, and says, 'it is possible that for studying the effect of substances in

solution, different frequencies of light, of varying light intensity and of temperature the method (bubble-counting method) may be put to use'. In our problem the effect of different colour of light and of varying light intensity was studied. Thus, according to Spoehr, the use of this method has some justification. Furthermore, the bubble-counting method possesses a distinct advantage over other methods in relation to this particular problem. By bubble counting the photosynthetic activity can be measured in very short time-intervals, even down to a few seconds. In our experience the change in the photosynthetic activity due to light change is a very rapid phenomenon, which lasts only about one or two minutes. When the measurements are taken at longer intervals this initial effect is entirely obscured. That the bubble-counting method measures photosynthetic activity at short time intervals was a decided advantage in our study. This was one of the important reasons which led the author to choose this method. Probably Warburg's (7) method of measuring the partial pressure of oxygen in a confined space with a differential manometer may serve the same purpose, but the ordinary gas-analysis method, which is generally considered as accurate, would not answer our purpose.

However, we must also realize that the results thus obtained are not strictly quantitative data. For even if we assume that the size of bubbles is constant, and the percentage of the oxygen in the bubbles is constant, the oxygen used in respiration and in diffusion directly to the water is not accounted for in our results. It is only an apparent rate of photosynthetic activity. Although our results cannot bring out the absolute change in photosynthetic activity, they are sufficiently accurate to bring out the trend of change in the photosynthetic activity due to light change.

In bubble counting two systems were employed. In the 1927 experiments we counted all bubbles which appeared every 10 seconds. One experimenter counted the bubbles while the other read the time. In the 1928 experiments we measured the time required for the emission of a certain number of bubbles in every 30-second period; this gave a more exact measurement.

III. Results.

1. *The Initial Effect of Alternation of White Light and Coloured Light, and the Alternation of Different Coloured Lights.*

In the first series of experiments fifty-six plants were studied. Eight of them are *Potamogeton* and the rest were *Myriophyllum*. They were subjected to various changes in colour of light. The results were rather diversified. Five types of initial response were recorded. They will be discussed in detail in the following paragraphs.

(a) *The First Type.*

The first type of response is best represented by Plant no. 2. Its response to the light change is presented in graphic form in Fig. 1. This type of response is similar to that observed in *Elodea* in our preliminary work. When the plant is transferred from white to coloured (red 2) light the photosynthetic rate drops down immediately. In this particular case

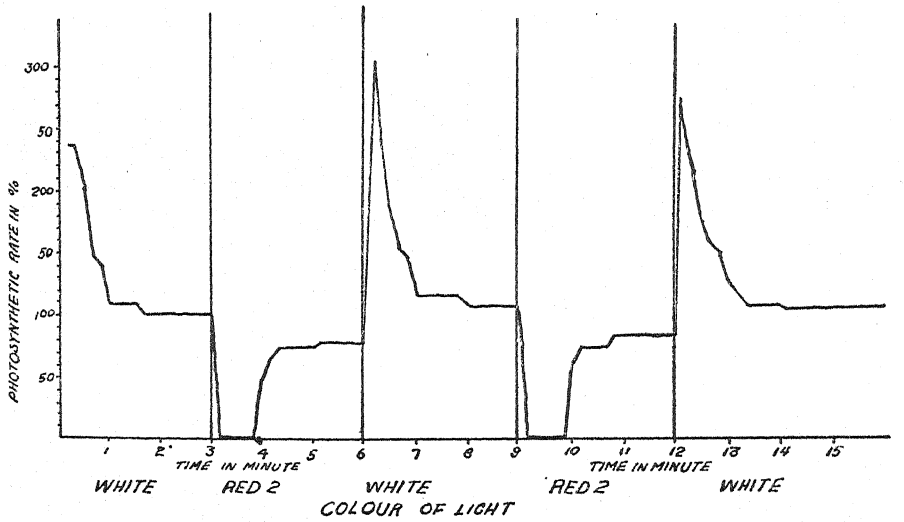


FIG. 1. Photosynthetic curve of Plant no. 2 (*Myriophyllum*) under alternation of red 2 and white lights. The ordinate represents the photosynthetic rate as a percentage based on the constant photosynthetic rate under white light as 100.

the photosynthetic activity drops down so low that the plant ceases to emit any bubble. Then it increases gradually to the normal and constant rate under coloured light. This is termed the initial inhibition effect. When the change of light is from coloured light of broader spectral range to coloured light of more limited range the same effect is observed. When the light is changed back from coloured to white a sudden jump is observed on the graph. In Plant no. 2 the number of bubbles emitted in the first ten seconds following the change is four times as high as that in red light. Then it is slowed down gradually to a constant rate, which is only about one-third of the initial rate. This is termed initial accelerating effect. The change from coloured light of limited spectral range to coloured light of broader range produces the same effect.

(b) *The Second Type.*

The second type of response can be represented by Plant no. 24 (*Myriophyllum*) under alternation of green and white light. The results are presented in graphic form in Fig. 2. From white light to coloured (green)

light the first bubble appears 150 seconds after the change. Then the rate increases gradually until it becomes constant. The initial prohibiting effect is very pronounced. From coloured to white light the photosynthetic activity of the plants rises to a constant rate immediately after the change.

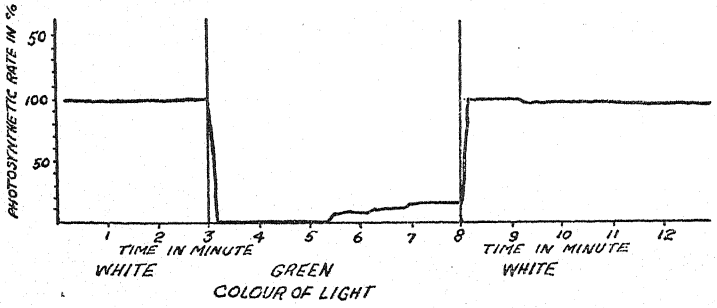


FIG. 2. Photosynthetic curve of Plant no. 24 (*Myriophyllum*) under alternation of green and white lights. The ordinate represents the photosynthetic rate as a percentage based on the constant photosynthetic rate under white light as 100.

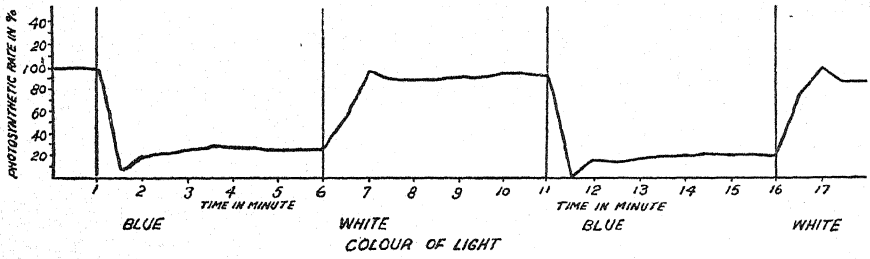


FIG. 3. Photosynthetic curve of Plant no. 51 (*Myriophyllum*) under alternation of blue and white lights. The ordinate represents the photosynthetic rate as a percentage based on the constant rate of photosynthesis under white light as 100.

There is no jump on the graph as recorded in Plant no. 2. Similar effect is observed by Bose in his second experiment on the initial effect of increasing light intensity. This type is termed the inhibition and instantaneously rising type.

(c) *The Third Type.*

The third type is best represented by Plant no. 51 (*Myriophyllum*) under the alternation of blue and white light. Fig. 3 is a graphic presentation of the results. From Fig. 3 we perceive that the same initial prohibiting effect is produced on changing from white to coloured light. But the effect due to the change of coloured to white light is different from both of the types described above. The photosynthetic activity rises gradually from the rate in the coloured light to a constant rate in white light. This type is termed the inhibition and gradually rising effect.

(d) *The Fourth Type.*

The fourth type of response is represented by Plant no. 37 (*Myriophyllum*) under the change of various light conditions. The data of the effect of alternation of red 1 and white light is presented in the continuous graph in Fig. 4. The change from coloured to white light produces the same response as in type 3, a gradually rising effect. But the most interesting

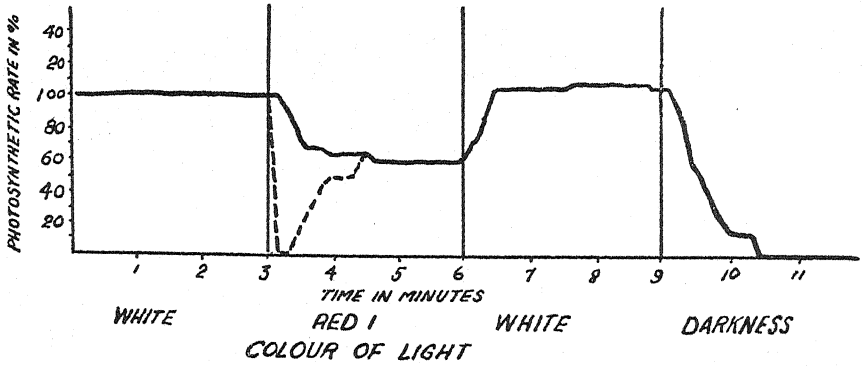


FIG. 4. Photosynthetic curve of Plant no. 37 (*Myriophyllum*) under alternation red 1 and white lights. The ordinate represents the photosynthetic rate as a percentage based on the constant photosynthetic rate under white light as 100.

fact is that in the change from white to coloured light no initial prohibiting effect is produced. The photosynthetic activity in the first ten seconds following the change to red light maintains the same rate as in the white light. Then it gradually drops down to a constant rate.

In the course of experimentation the author observed another interesting phenomenon. After the light was turned off bubbles were still emitted from the cut end of the plants. In others the bubble emission ceased directly after the light was cut off. The number of bubbles emitted per unit time in successive periods after cutting off the light was recorded and plotted on Fig. 4. The rate of bubble emission maintained the same rate in the first ten seconds immediately after cutting off the light. Then the rate gradually dropped down to zero. The probable explanation of the phenomenon will be given later. This discovery brought the author to realize that the photosynthetic graph in coloured light in Fig. 4 represents the combined effect of the two factors. In order to isolate the real photosynthetic activity of the coloured light we have to deduct from the apparent rate the value of photosynthetic activity at the corresponding dark period. The results are plotted in the dotted line in Fig. 4. The initial inhibition effect is again prominently brought out. The fourth type is thus termed the modified initial inhibiting and gradually rising effect.

(e) *The Fifth or Intermediary Type.*

The fifth type of response is characterized by a constant inhibiting effect due to the change from white to coloured light. The effect due to the change from coloured light to white light is variable. An accelerating effect and instantaneously rising effect may be observed in one plant in two successive experiments, and instantaneously rising and gradually rising effects in another. We term this the intermediate type.

(f) *The Relative Importance and Interrelation of Different Types.*

The results of all the experiments performed under various kinds of light alternations are collected in Table I. On examining this Table carefully one will observe the fact that the prohibiting and accelerating type

TABLE I.

The Initial Effect caused by Colour-changes of Light.

Light Alternation.	Species.	Number of plants showing— Inhibiting and accelerating effect.	Inhibiting and instantaneously rising effect.	Inhibiting and gradually rising effect.	Modified inhibiting and gradually rising effect.	Intermediary type.
Red 3 and white	*Myriophyllum*	5	—	—	—	—
	Potamogeton	2	—	—	—	—
Red 2 and white	*Myriophyllum*	27	—	1	3	1
	Potamogeton	2	—	—	—	—
Red 1 and white	*Myriophyllum*	16	—	1	3	1
	Potamogeton	5	—	—	—	—
Blue and white	*Myriophyllum*	2	1	1	2	—
Green and white	*Myriophyllum*	1	2	1	3	—
Red 2 and red 3	*Myriophyllum*	8	—	—	—	—
	Potamogeton	2	—	—	—	—
Red 1 and red 2	*Myriophyllum*	12	—	—	—	1
	Potamogeton	2	—	—	—	—
Blue and red 2	*Myriophyllum*	1	—	—	—	—
Green and red 2	*Myriophyllum*	—	—	—	—	1
Blue and red 1	*Myriophyllum*	3	—	—	—	—
Green and red 1	*Myriophyllum*	1	—	—	—	—

of response is the prevailing type of both species. In fact, in *Potamogeton* no other type of response is observed. All other types can be said to be modifications of the prevailing type. A careful analysis of the magnitude of accelerating effect in various specimens under the first type substantiates our contention. By the magnitude of accelerating effect we mean the increased rate of bubble emission in the first ten seconds after the change to white light over the constant rate of bubble emission in white light. It is expressed in percentage, taking the constant rate in the white light as 100. In *Myriophyllum* it varies from 232 per cent. in Plant no. 23

to 11 per cent. in one experiment on Plant no. 6, when the light changed from red 2 to white. In *Potamogeton* it varies from 12 per cent. in Plant no. 48 to 80 per cent. in Plant no. 46, under the same light change. Between these two limits many different intermediate forms exist. Now suppose we decrease the magnitude of the accelerating effect to zero, then the plant is classified under the second type, or the initial inhibition and instantaneously rising type. If this value is further decreased to a minus value, it is then classified under the third and fourth types. Then the second, third, and fourth type of responses are the extension of the lower limit of the first type, and the plants showing those responses are in the lower portion of the range of natural variation. The existence of the intermediary type strengthens our claim. The probable cause of this type of variation will be discussed later.

It is also interesting to note that one plant may show different types of initial effect under different kinds of light change. This is well illustrated in Plant no. 24. It shows an acceleration of 29 per cent. in the change from blue to white, and shows an instantaneously rising effect in the change from green to white light.

(g) *The Changes in Light.*

In order to determine whether the *colour* of the light is the responsible factor, causing the initial effects described above, we have to examine the alternating lights more critically. In using the coloured filter, the filtered light is not only changed in colour but also in intensity and so in the amount of available energy. The foregoing data are not conclusive to prove that the colour change causes the initial inhibition and acceleration effect.

2. *The Initial Effect of Alternations of Light of Different Intensity.*

In the second series of experiments five plants are employed, two of *Ceratophyllum*, two of *Potamogeton*, and one of *Myriophyllum*. All of them show uniform response to the change of light intensity. Plant 78 is chosen to represent the type. The results are presented in graphic form in Fig. 5. The graph distinctly shows that when a plant is shifted to an increased light intensity an accelerating effect is produced, and to a decreased light intensity an inhibiting effect is produced. Our results apparently differ from Bose's result. He found that one *Hydrilla* plant showed a gradually rising effect when the light was increased from 750–1000 lux, and an instantaneously rising effect when the light was increased from 1000–1500 lux. From the discussion in the previous section it is clear that Bose accidentally chose an extreme specimen.

From the results of the second series of experiments it seems to indicate that light intensity is the factor. But in these experiments only

the colour of light is kept constant. There are still two variables. They are light intensity and available energy. One may think that light intensity and available energy are two terms expressing the same thing. However, these two can be distinctly differentiated. The amount of light energy available to the plant depends upon four factors: firstly, chlorophyll absorption, secondly, the energy of the absorbed rays, thirdly, the effectiveness of the absorbed light, and lastly, light intensity. Chlorophyll absorption and the amount of energy vary with the wave length of the rays. These are well-known facts. The effectiveness of the absorbed light also varies. According to Warburg and Neglein (10) it is directly proportional to the wave length. Thus the amount of available energy of green light is very different from that of a red light of the same intensity.

3. *The Initial Effect and its Relation to Alternations of Different Coloured Lights with the same amount of available Energy.*

In the third series of experiments ten plants were tested, six *Myriophyllum*, two *Ceratophyllum*, and two *Potamogeton*. They were under two sets of experimental conditions, the first between the orange-coloured light and white light, and the second between red 2 and white light. Uniform results were obtained. These may be illustrated with Plant no. 65 in Fig. 6. A slight depression is recorded both when the change is from white to coloured light and from coloured to white light. The magnitude of depression was about the same in both cases. This slight depression was thought to be due to the change of position in relation to radiation. In our experiments the two lights came from opposite sides of the plants. Furthermore, when the light was switched from one to another there was a slight period of darkness of about a fraction of a second, which caused a slight depression in photosynthetic activity. The characteristic acceleration and inhibition effect was not recorded. In this series of experiments the alternating lights differed both in intensity and in colour, but with the same amount of available energy. The intensity was greater in the white light than in the coloured light.

4. *The Determining Factor.*

The results in all three series are tabulated in Table 2. From the table it is clear that there are three types of changes due to light alternations in the various series of experiments, i. e. change in colour, change in intensity, and change in the amount of available energy. In order to determine which of these three factors is responsible for this initial effect, each of the middle three columns is compared with the fifth column. When the colour-change column is compared with the initial-effect column no correla- is found. The light change from white to coloured produces an initial

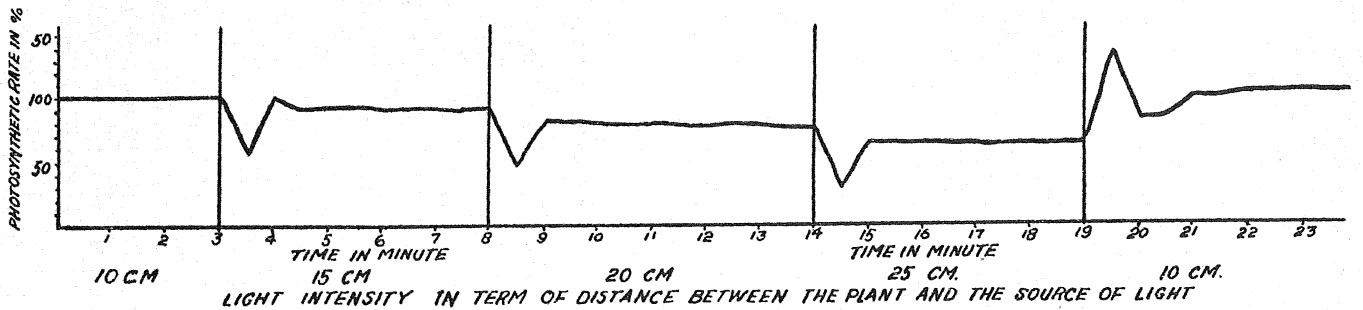


FIG. 5. Photosynthetic curve of Plant no. 78 (*Myriophyllum*) under the change of light of various intensities. The ordinate represents the photosynthetic rate in percentage based on the constant photosynthetic rate under light of strongest intensity (distance between plant and light source is 10 cm.) as 100.

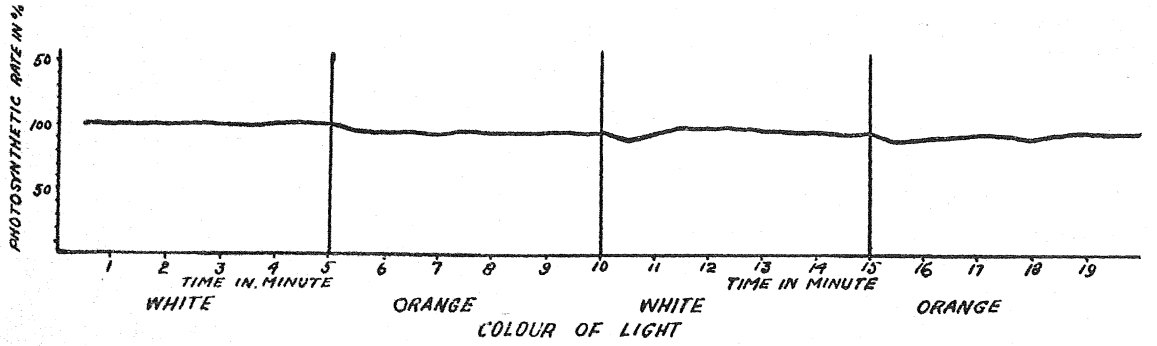


FIG. 6. Photosynthetic curve of Plant no. 65 under alternation of lights of different colour but with the same amount of available energy. The ordinate represents the photosynthetic rate in percentages based on constant photosynthetic rate under white light as 100.

accelerating effect in the first series and no effect in the third series. At the same time both acceleration and inhibition effects appear in the second series while the colour of light remains unchanged. Thus it is safe to exclude the colour-change from consideration. Next the intensity-change column is compared with the initial-effect column, there again we failed to find a perfect correlation. The results of the first and second series agree with each other, both give an initial accelerating effect on the increased intensity and an inhibiting effect on decreased intensity, but in the third experimental series this agreement fails; no accelerating effect is produced in decreased intensity. Thus the intensity change is also excluded. Then there remains only one factor, the change in the amount of available energy for photosynthetic activity, which must be the principal cause of the production of the characteristic initial acceleration and inhibition effect. A comparison of column 4 with column 5 substantiates our contention: there is a perfect correlation between these two columns—the increased photosynthetic available energy invariably produces an initial accelerating effect and the decreased available energy an initial prohibiting effect. No pronounced effect is produced when the amount of available energy remains constant in spite of changes in other light factors.

TABLE II.

The Relation between Light-changes and the Initial Effect.

Experimental Series.	Light Change.	Intensity.	Available Energy.	Initial Effect.
1.	Coloured–white	Increased	Increased	Acceleration
2.	No change	Increased	Increased	Acceleration
3.	Coloured–white	Increased	No change	None
1.	White–coloured	Decreased	Decreased	Inhibition
2.	No change	Decreased	Decreased	Inhibition
3.	White–coloured	Decreased	No change	None

IV. DISCUSSION.

The results presented in the foregoing sections bring out a few interesting facts, which seem to throw some light on the modern concept of the mechanism and of the chemistry of photosynthesis. In 1922 Warburg and Neglein (9) suggested that photosynthesis takes place only when the surface of chlorophyll is in contact with the molecule of carbonic acid. Immediately after photosynthesis the surface is covered with carbohydrates, which take time to diffuse away. During this period the particular surface is deprived of the chance of being in contact with the carbonic acid. The surface again becomes available for such contact after the carbohydrates diffuse away. From the point of view of effectiveness the surface is constantly destroyed and recovered. As a rule, when a plant attains a constant rate in photosynthetic

activity, the surface-destruction rate and the rate of recovery are in an equilibrium. Baly and his associates (1, 2, 3) have actually proved the importance of the surface by experiments in the artificial photosynthesis of carbohydrates from carbonic acid both in ultra-violet and visible lights. They also believe that surface is constantly destroyed and constantly recovered; but they consider oxygen as the destroying agent instead of carbohydrates. However, oxygen and carbohydrates are both photosynthetic products. The present results strongly support the theory of the surface mechanism of photosynthesis. The initial accelerating effect in the light of increased available energy and the initial prohibiting effect in the light of decreased available energy are mainly due to a change in the extent of the available surface.

When the plant is suddenly shifted to a light with increased available energy the photosynthetic activity immediately jumps up on account of the increased energy received by the surface. The rise of photosynthetic activity produces a corresponding rise in photosynthetic product. Thus the rate of surface destruction rises far above the rate of surface recovery. The equilibrium is affected. The area of available surface decreases and the area of non-available or destroyed surface increases. The decrease in the former automatically causes a drop in photosynthetic activity in spite of the unchanged amount of available energy. This explains the later drop on the graph after a sudden rise. The increase in the non-available surface brings about an increase in surface recovery; it will rise continuously, but with diminishing rate. At the same time the surface-destroying rate drops down gradually, due to the drop in photosynthetic activity after the abrupt rise. An equilibrium is thus finally attained. The area of available surface becomes constant without further decrease and the photosynthetic activity attains a constant rate.

In the same manner we can explain the inhibition effect in the light of decreased available energy. Directly after the change the photosynthetic activity drops down very low, due to the limited area of available surface. This drop brings about the decrease in the surface-destroying rate and consequently an increase in the area of available surface. The increase of the latter will bring about the rise in photosynthetic activity. This explains the rise after the abrupt drop seen on the graph. The surface-destroying rate following the photosynthetic activity of the plant rises gradually after the sudden drop. At the same time the surface-recovering rate decreases gradually on account of the decrease in non-available or destroyed surface. Finally an equilibrium is established. The area of available surface becomes constant without further increase. This explains the fact that the photosynthetic activity rises in a form of gradual slope until it reaches a constant rate after the abrupt drop.

In the foregoing discussion only the photochemical reaction is con-

sidered in the complicated process which we term photosynthesis. This may be effected by other reactions in the same process. It is generally considered that the splitting off of oxygen does not take place in the primary photochemical reaction. Usher and Priestley (6) assume that it is an enzymic reaction, and recently Willstätter and Stoll (11) put forward the same view. Warburg (7, 8) terms it an automatic reaction. The results from our study seem to prove from another angle the validity of this general contention. As we have noticed in Plant no. 37 the oxygen emission does not stop with the exclusion of light. From Fig. 4 we know it lags behind more than 80 seconds. This clearly indicates that the splitting off of oxygen is not a photochemical reaction but a reaction which follows the photochemical one. It may be enzymic as stated by Priestley, Willstätter, and their associates.

From our data it appears that the degree of co-ordination of these two reactions varies with individuals. Some individuals show better co-ordination than others. Plant no. 37 is an extreme case, the lag of the second reaction amounts to about 80 seconds. Plants nos. 7 and 8 only show a lag of 10 seconds. However, no appreciable amount of lag is noticed in the majority of the experimental plants.

In view of this fact, we can easily explain the occurrence of the instantaneously rising effect and the gradually rising effect in the light of increased available energy. These generally occur in the individual with a slower enzymic reaction. Plants nos. 7, 8, and 37 show a typical gradually rising effect. Our results based on oxygen emission are only a measure of the rate of enzymic reaction. If the enzymic reaction maintains the same or a faster rate than that of photochemical reaction, the effect of the latter will be fully brought out. This condition is probably attained in Plants no. 23 and no. 2. They show an acceleration of a magnitude of more than 200 per cent. But when the enzymic reaction is slower than the photochemical reaction, an abrupt rise in the rate of the latter is prevented, due to the accumulation of photochemical products. By the mutual adjustment of these two reactions an instantaneously rising or a gradually rising effect results.

V. Summary.

An initial inhibition effect on photosynthetic rate is observed when the plant is changed from the light of high available energy for photosynthesis to that of low available energy. An initial acceleration effect is observed when the plant is shifted from the light of low available energy to that of high available energy.

These effects are observed in plants of the following genera: *Elodea*, *Myriophyllum*, *Potamogeton*, and *Ceratophyllum*.

This phenomenon strongly supports the theory of surface action in photosynthesis, postulated by Warburg, Baly, and their associates.

The result of the present investigation also indicates that splitting off of oxygen takes place not in the primary photochemical reaction but in a separate later reaction, which may be an enzymic one. Furthermore, these reactions may not have a concordant rate. The degree of correlation of these two reactions varies with individual plants. In a plant which is characterized by a slow enzymic reaction the initial inhibition and acceleration effects may be entirely obscured.

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The Chromosome Numbers of Indian Cottons.

BY

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THE importance of cytological studies in genetical investigations has been recognized in recent years. For a proper understanding of the mechanism of heredity a cytological knowledge of the material is very desirable. This is all the more important in an economic crop like cotton where, as is well known, the American or Egyptian cottons do not cross freely with the Indian types, and as such the chances of bringing about a desirable combination are very remote. Zaitzev (1) in Russia and Desai (2) in India claim to have brought about such a combination, but both report that the conjugate generation plants were completely sterile. Denham's (3) investigations on the cytology of the cotton plant show that the American, Egyptian, and Indian cottons fall into two distinct groups, the first two being characterized by double the number of chromosomes occurring in the latter. He suggests that this might perhaps explain the impossibility of crossing American or Egyptian cottons with the Indian types. The fact that chromosome numbers range in multiples of some basic number in the same genera has also been noted in other crops. Gaiser (4) states that the haploid chromosome number of *Triticum* and *Avena* are 7, 14, and 21, of *Nicotiana* 9, 12, 18, and 24; other cases are also on record. Interspecific crosses between these different groups have led to considerable sterility. As a rule the greater the difference in the chromosome numbers the greater the degree of sterility.

As Indian cottons differ considerably in their morphological and other characters, it was thought that a critical cytological study might reveal certain strains with a higher chromosome number than that obtained by Denham (3) for the Indian cottons. Besides, it would enable us to have a detailed knowledge of the chromosome numbers of Indian cottons and add further evidence to that obtained by previous workers on the chromosome numbers of the Old and New World cottons. With this in view the present work was undertaken.

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Material and Methods.

The material used in this investigation has been grown from selfed seeds obtained from various sources. Some of the material has been collected personally from different cotton-breeding centres in India. All the types examined cytologically have been grown from pure lines selfed over a number of years. Of the thirty-two types worked out, four are acclimatized American cottons which are widely cultivated in India for their superior qualities, the rest are indigenous Indian cottons of varying merit.

Two fixatives were first tried, Flemming's weak fluid and Allen's modified Bouin's fluid, the formula of which is given below.

Picric acid saturated aqueous solution	75 c.c.
Formalin (commercial)	25 c.c.
Glacial acetic acid	5 c.c.
Urea crystals	2 grm.
Chromic acid crystals	1·0 grm.

Fixation was very poor with Flemming's fluid. There was considerable shrinkage of the material and no detail was obtained. Bouin's fluid, on the other hand, gave very good results, and as such was exclusively used later. Collections of material were usually made on bright days between 12 and 4 p.m. Buds of various sizes ranging from 1·5 to 4 mm. in diameter were fixed. Those of larger size generally contained fully formed pollen grains or were in the tetrad stage. To facilitate penetration of the fluid, the bracts, perianth, and the ovary were completely removed before fixation. The fixing fluid was heated up to an initial temperature of 40° C. before fixation, and allowed to cool as material was collected. The material was kept in the fixing fluid for a period of twenty hours, after which it was run up to 70 per cent. alcohol in the course of an hour, and repeatedly washed in 70 per cent. alcohol every twelve hours till the green coloration disappeared. It was then dehydrated by passing through successive higher grades of alcohol, de-alcoholized in several ascending series of xylols, and imbedded in paraffin. Sections were cut 8 μ thick.

Flemming's triple strain and Heidenhain's iron-alum haematoxylin were used, the latter was found to be excellent for counting chromosomes.

The chromosomes were counted from the meiotic stages of the microspore mother cell. Check counts were taken from several buds to eliminate error due to abnormal pollen formation. Haploid chromosome number has been represented in all cases.

Types Examined.

	Chromosome number.
1. *The Acclimatized American Cottons.*	
Gossypium hirsutum, Mill.	
C. O. I. Seeds obtained from the Cotton Specialist, Government of Madras	26
440. Material obtained from Government Experiment Station, Dhulia. A Coimbatore selection of Cambodia cotton	26
4 F. A Punjab selection of American cotton	26
Buri. An American cotton selected and grown widely in Central Provinces	26
2. *The Herbaceum Cottons.*	
Gossypium herbaceum, Linn.	
Hagari 25. Seeds obtained from the Cotton Specialist, Government of Madras	13
Kumpta. Seeds of this and the next variety were obtained from the Cotton Botanist, Mysore Department of Agriculture	13
Mysore Local (Naked seeded). Classified by Gammie as *Gossypium herbaceum*, var. nov. *melanosperma*	13
Dharwar I. Seeds obtained from the Government Experiment Station, Dharwar	13
Seeds of the following were obtained from the Bombay Department of Agriculture, Poona:	
Broach. No. 6 (Wilt resistant)	13
Goghari. (A. 26)	13
Wagad. (8)	13
1027. *A. L. F.*	13
I. A. Cylindrical Boll	13
I. A. Long Boll	13
3. *The Neglectum Cottons.*	
Gossypium neglectum, Tod.	
G. neglectum, var. nov. *rosea* (Gammie). Material obtained from the Government Experiment Station, Dharwar	13
G. neglectum, sub. var. nov. *malvensis* (Gammie). Seeds of this and the following were sent by the Economic Botanist to the C. P. Government, Nagpur	13
G. neglectum, var. nov. *vera* (Gammie)	13
G. neglectum, sub. var. nov. *cutchica* (Gammie)	13
G. neglectum, sub. var. nov. *Kathiavarensis* (Gammie). Materials for this and the next were obtained from the Government Experiment Station, Dhulia	13
G. neglectum (Naked seeded)	13
W. N. 27. A selection of *neglectum roseum.* Seeds sent by the Cotton Botanist, Sind.	13
Wagale (Yellow flowered). Material obtained from the Government Experiment Station, Dharwar	13
Wagale C. 19 (Pale yellow-flowered). Isolated by the Cotton Breeder, Dhulia. Material obtained from that Station	13

	Chromosome number.
4. *The Indicum Cottons.*	
Gossypium indicum, Lamk.	
Nandayal 14. Seeds obtained from the Cotton Specialist, Government of Madras	13
Bani (Hyderabad). Seeds sent by the Department of Agriculture, Hyderabad	13
Bani (Nagpur). Seeds obtained from the Economic Botanist to the C. P. Government, Nagpur	13
Mungari 274. A Madras selection. Seeds obtained from the Government Experiment Station, Hagari	13
G. Indicum, var. nov. *Mollisoni* (Gammie). Seeds obtained from the Punjab Department of Agriculture	13
5. *Other Cottons.*	
Nadam (*G. obtusifolium*, Gammie). Seeds obtained from the Cotton Specialist, Government of Madras	13
Cernuum (*G. cernuum*, Tod.). Material obtained from the Government Experiment Station, Dhulia	13
G. arboreum, Watt. Seeds of this and the next were sent by the Cotton Botanist to the Government of Punjab, Lyallpur	13
G. arboreum, var. *sanguinea*, Watt	13

The above list confirms Denham's investigations and leaves no doubt about the chromosome numbers of Indian cottons. The Arboreum and Nadam cottons, which differ considerably in their characters from the rest of the Indian cottons and which are grown as perennials in some parts of India, failed to reveal any difference from the typical chromosome number (13) of the Indian cottons. The Herbaceum and Neglectum groups also showed no variation in this respect. The wild cotton of India—*G. Stocksii* of Sind—which has been examined by Youngman and Pande (5), also showed thirteen chromosomes in the reduction division. It is very doubtful if there is any pure Indian cotton which has a higher number of chromosomes, but nothing definite can be said till all the Burmese cottons have been examined critically.

No appreciable difference in the size of the chromosomes was noted, though in some cases in the Indian cottons one slightly bigger chromosome was met with. The difference in the size and shape of the chromosomes (which are very small) might be explained by the fact that while in some sections parts of chromosomes are represented, in others the entire chromosomes are present.

An increase in the chromosome number is often brought about by crossing. This has been noted by Clausen (6) and others. It should be mentioned, however, that in these cases the parents differ in their chromosome numbers. The hybrid origin of these plants with a higher chromosome number could be made out by a careful cytological study. The irregular

meiotic divisions, and the indications of sterility are the determining factors. Certain Indian cottons show a certain amount of sterility, but no irregularities in the reduction division were noted except in the occurrence of a few sterile anthers.

SUMMARY.

The chromosome numbers of twenty-eight Indian and four acclimatized American cottons have been determined. The American cottons were found to contain twenty-six chromosomes and the Indian thirteen (Haploid). This agrees with the numbers obtained by Denham (3) for the Old and New World cottons.

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Notes on Conifers.

II. Some Points in the Morphology of Larix europaea DC.

BY

W. T. SAXTON.

With sixteen Figures in the Text.

SINCE the time of Hofmeister and Strasburger numerous botanists have interested themselves in the morphology of the Larch. In spite of this various points in its morphology appear never to have been accurately recorded. No attempt will be made here to traverse the rather extensive literature dealing with the genus, reference being made only to such papers as have a direct bearing on the features discussed.

On account of the very early development of the spore-mother-cell in *Larix* the position of the ovule is definitely fixed even before that organ is externally visible, and when the nucellus is first recognizable it projects laterally at right angles to the ovuliferous scale. In median radial section the young ovuliferous scale appears at this time as an undifferentiated mass of tissue axillary to the bract (Fig. 1). A section parallel to the preceding may cut transversely through the nucellus (Fig. 2), while a tangential section may pass through both megasporangia, as in Fig. 3, and a transverse section (Fig. 4) also shows both spore-mother-cells. So far as the writer is aware there is no previous record of this early position of the ovules, though it is probable that Strasburger must have been acquainted with it, since he has described the early development of the ovule (Strasburger, 4). The mature position, with both ovules on the upper surface of the scale and with the micropyle facing the axis, is reached through differential growth of the scale, especially through rapid growth of the lower and outer sides at about the time of pollination, and is, of course, familiar, and characteristic of all Pinaceae. In discussing the brachyblast theory Masters (3) made the pertinent criticism that in fact no one had ever observed the ontogenetic movement of ovules which should occur if the ovuliferous scale is equivalent to the short shoot of a two-needled Pine with the ovules developed on the lower (dorsal) surface of each needle. That criticism has been met for the first time in the facts recorded above, which appear to afford substantial support to the brachyblast interpretation of the cone in Pinaceae.

[Annals of Botany, Vol. XLIII. No. CLXXI. July, 1929.]

Several authors have studied microsporogenesis in the Larch, and among them Allen (1) and Devisé (2) have paid special attention to the development of spindle fibres. It seems relevant to point out that all their

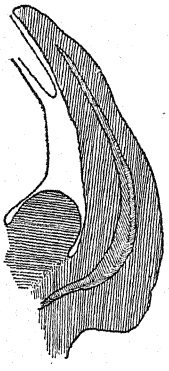


FIG. 1.

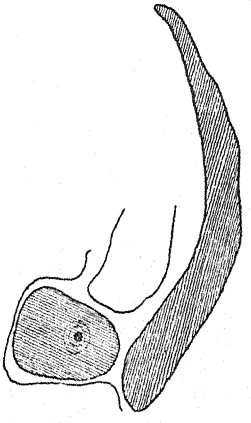


FIG. 2.

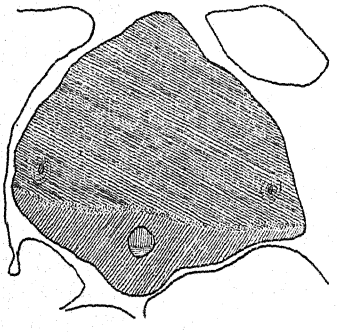


FIG. 3.

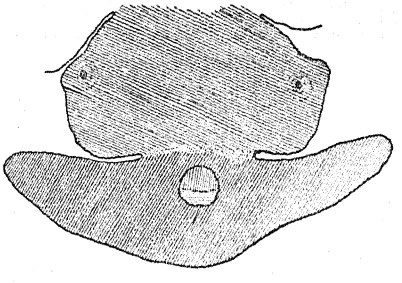


FIG. 4.

FIGS. 1–4. 1. Median radial section through a bract scale and its axillary ovuliferous scale. × 75. Oct. 17, 1928. 2. Section parallel to the preceding, passing transversely through a very young ovule. × 75. Oct. 17, 1928. 3. Tangential section through bract scale and ovuliferous scale, passing through both ovules. × 75. Oct. 17, 1928. 4. Section through the bract scale and ovuliferous scale, transverse to the cone axis and in the plane passing through both ovules. × 75. Oct. 17, 1928.

(Figures 1 to 4 are semi-diagrammatic, and the ovules are from cones similar to that shown in Fig. 13.)

figures are drawn from *late stages* of the heterotype division. The spore-mother-cells pass through synapsis towards the end of September and after recovery remain for months in a quiescent state before completing the division. A study of synaptic and early post-synaptic stages has indicated a possible origin of spindle fibres different from, and very much earlier than, those postulated by either Allen or Devisé. Figures 5 and 6 show the appearance in synapsis, a coil or bundle of fibres appearing at one end of the cell, very suggestive of spindle fibres. This coil is quite conspicuous

and can be seen easily under a low power in a large proportion of the cells. Figures 7 and 8 are drawn from post-synaptic stages, where the coil has apparently divided into two, now found on opposite sides of the cell. The

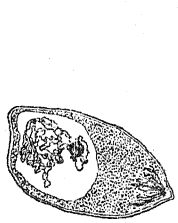


FIG. 5.



FIG. 6.

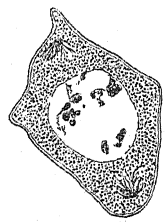


FIG. 7.

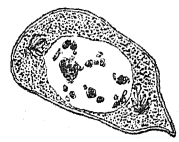


FIG. 8.

FIGS. 5–8. 5 and 6. Two microspore-mother-cells in synapsis, showing a structure interpreted as probably the first appearance of spindle fibres. × 500. Sept. 24, 1928. 7 and 8. Two microspore-mother-cells showing a stage soon after recovery from synapsis. The fibres now appear on both sides of the cell. × 500. Oct. 11, 1928.

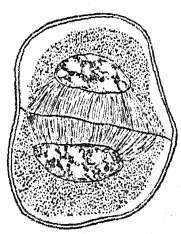


FIG. 9.

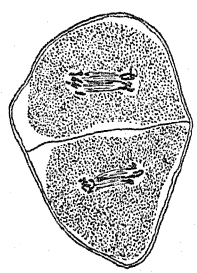


FIG. 10.

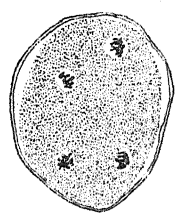


FIG. 11.

FIGS. 9–11. 9 and 10. Microspore-mother-cells showing the formation of a permanent wall after the first division. × 500. Feb. 2, 1929. 11. Microspore-mother-cell showing complete absence of walls, although the second division has already taken place. × 500. Feb. 2, 1929.

very definite position of the coils in the two stages seems to make it most unlikely that they are merely artifacts, although such an explanation is not, perhaps, wholly precluded, as, though a cytological fixative was used, the proportion of acetic acid in it was rather high.

Devisé (2) remarks (and similar opinions are expressed or implied by various authors) that no wall follows the first division of the pollen-mother-cell in any Conifer. His own figures of *Larix* show the disappearance of the incipient wall in every case. In point of fact the formation of a permanent wall undoubtedly takes place at the first division in *Larix* in some cases, while in others it may be delayed until after the second division. Figs. 9 and 10 show examples of the first method, Fig. 11 of the second.

Devisé also figures the breaking up of the whole spore-mother-cell into spores, not within the spore-mother-cell wall. It is doubtful if such a method of microspore formation takes place in any Conifer, and certain that it is not the normal method in the Larch. Out of many hundreds of

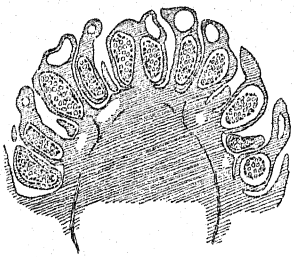


FIG. 12.

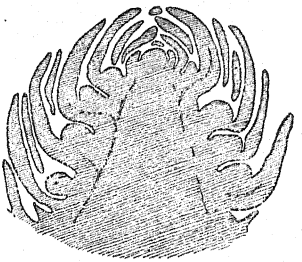


FIG. 13.

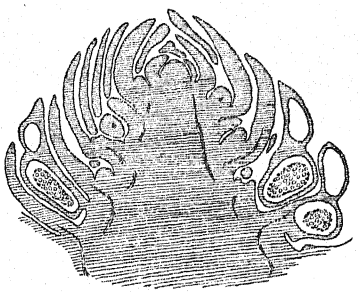


FIG. 14.

FIGS. 12–14. 12. Median longitudinal section of a male cone gathered in winter. × 15 Nov. 15, 1927. 13. Median longitudinal section of a female cone gathered in winter. × 15. Feb. 9, 1929. 14. Median longitudinal section of a bi-sporangiate cone of the same age as the preceding. × 15. Feb. 9, 1929.

(The bud-scales have been removed in Figs. 12–14.)

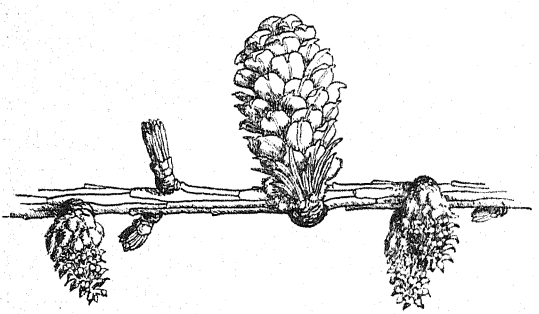


FIG. 15.

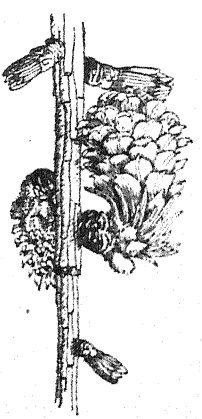


FIG. 16.

FIGS. 15 and 16. 15. Sketch of part of a horizontal branch showing erect female and pendulous male cones. Nat. size. April 6, 1929. 16. Similar sketch of part of a pendulous branch. Nat. size. April 6, 1929.

spore-mother-cells examined[1] at the stage when this point could be ascertained the spores were seen to be formed within the spore-mother-cell wall in every case, though, as usual, the mother-cell wall very quickly breaks down and disappears after the spores have been formed.

Unlike the Pine, male and female cones of the Larch are borne in identical positions (terminally on short shoots) and it is practically impossible to distinguish one from the other until a few days before they burst through the bud-scales, when the slightly more pointed apex of the female cone makes it recognizable to an experienced eye. The same distinction is far more obvious when the cones are cut in half lengthwise or seen in section, as indicated in Figs. 12 and 13 which are drawn from longitudinal sections of cones gathered in mid-winter. Bi-sporangiate cones are rare; one of two collected by the writer is shown in section in Fig. 14.

Until the cone-buds burst they show no indication whatever of any geotropic curvature, but as soon as they grow out of the bud-scales the female cone becomes negatively and the male positively geotropic, with the result that in a few days time every female cone is erect and every male cone pendulous, as recorded by Goebel (Morphologische und biologische Bemerkungen, 13; Flora, xci. 237–255, 1902). This is seen in Figs. 15 and 16 in horizontal and pendulous branches respectively. Bi-sporangiate cones are negatively geotropic, like the females.

All the drawings are from *L. europaea* var. *pendula* and were kindly made for me by Miss L. E. Hawker, to whom my thanks are due.

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[1] By crushing sporangia in a drop of aceto-carmine.

Studies on the Transport of Nitrogenous Substances in the Cotton Plant.[1]

II. Observations on Concentration Gradients.

BY

E. J. MASKELL

AND

T. G. MASON.

With two Figures in the Text.

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[1] Paper No. 4 from the Physiological Department of the Cotton Research Station, Trinidad.

[Annals of Botany, Vol. XLIII. No. CLXXII. October 1929.]

Section I. Introduction.

In Part I (8) an account was given of some preliminary work on the downward transport of nitrogen in the stem. It was indicated that a synthesis of organic nitrogen takes place in the leaves, and that nitrogen is exported thence via the stem to the roots. This downward movement of organic nitrogen occurs in the bark, while the upward migration of inorganic nitrogen is through the wood.

By day the rate of import of inorganic nitrogen by the leaf exceeds the rate at which organic nitrogen is exported ; by night the reverse obtains. As a result the leaves show diurnal variation in total nitrogen, the amount increasing by day and diminishing during the night. This diurnal variation in the leaf is reproduced in the bark of the main-axis. In the case of plants carrying a load of bolls no diurnal variation was, however, observed in the bark of the stem below the foliage region. When continuity of the bark tissues is interrupted by removal of a ring of bark, nitrogen accumulates above the ring not only in the bark but also in the wood and in the leaves. In the ringing experiment reported in Part I the accumulation in the leaves was not statistically significant, but further work has definitely established the existence of this response. It was also ascertained that if, instead of removing a complete ring of bark from the main-axis, only partial rings are made, leaving intact strips or bridges of bark of varying widths, the total amount of nitrogen transported downwards falls off as the bridge is made narrower, but the rate of movement across the unit cross-sectional area in the constricted region is increased. The gross phenomena of nitrogen transport thus show a striking similarity to the phenomena of carbohydrate transport (9, 10) ; in both cases the observations suggest that movement is by a process analogous to diffusion.

In our studies on the transport of carbohydrates, simultaneous observations on the amount transported and on the sugar gradients in the bark sap showed not only that downward movement takes place along a well marked concentration gradient, but also that changes in the steepness of the gradient are accompanied by similar changes in the rate of transport. The gross phenomena of carbohydrate transport, namely, the spread of diurnal changes from leaf to bark, the accumulation of carbohydrate in all the tissues above the ring, and the type of response shown to partial ringing, seem thus to be due to the fact that carbohydrates move as sugars and that the rate and direction of movement are determined by the concentration gradient.

The resemblance between the gross phenomena of carbohydrate and nitrogen transport suggests that nitrogen transport too, should be determined by the concentration gradients of some mobile form of nitrogen. In the present and ensuing papers on nitrogen transport the methods used in

studying concentrations and concentration gradients in carbohydrates are extended to nitrogen compounds. As in the case of carbohydrates a number of inter-related problems have to be settled before even a tentative picture of the mechanism of transport can be built up; in addition, the problem of nitrogen transport is further complicated by the fact that the type of chemical analysis possible in routine work does not generally permit of the estimation of definite compounds, but only of certain groups. The number of these groups is, moreover, greater than the number of compounds that required consideration in the case of sugars. An additional difficulty lies in the unknown composition of a considerable part of the crystalloid N of the plant.

The central problem may be stated as follows: Are variations in the rate and direction of longitudinal movement of organic nitrogen in the bark determined by longitudinal concentration gradients of the mobile form of nitrogen in the channels of transport? And, if so, is the acceleration in the rate of transport, as compared with diffusion *in vitro*, of the same order as that found for the transport of sugars? It will be seen that the solution of this problem involves the solution of a number of subsidiary problems, viz. (1) the channel of transport, whether the sieve-tubes or some other tissue, (2) the nature of the mobile compound or group of compounds, (3) some estimate of the concentration gradient of the mobile form in the channel of transport. With the technique available hardly one of these questions could be settled independently of the others. The problem resembles that of the solution of a set of simultaneous equations. Consequently, although certain experiments were designed to throw light mainly on one aspect or other of the whole problem, the data cannot well be presented under the separate headings outlined above. The method of presenting the data has accordingly been based rather on the type of observation made. In the present paper are grouped observations on the concentrations of nitrogenous compounds at different vertical levels in the bark, the wood, and the leaves, on the radial concentration gradients across the bark, and on the concentration gradients from the leaf lamina to the petiole. These observations indicate the normal context of concentration conditions for transport in the plant.

Section II. Methods.

(a) *Material.*

The same strain of Sea Island Cotton was used as in our work on carbohydrate transport. As before, vegetative-branches were removed so that the scaffolding consisted only of the main-axis and its fruiting-branches. With certain exceptions, which will be indicated later, the plants were devoid of bolls, the flower-buds having been cut off before the time of flowering.

(b) *Grading.*

Samples of plants for analysis were drawn after grading the field population; usually the grading was done on the basis of height measurements. Owing to the considerable volume of sap required for the estimation of nitrogen fractions a very large number of plants had to be taken in each sample. When two or more groups of differently treated plants were being compared it was not always possible, therefore, to handle at each collection the number of plants required for duplicate samples of each group; often only one sample from each group could be dealt with. In such cases the variation between different samples of the same group is due partly to sampling and partly to the effect of time. By the application of Fisher's analysis of variance (6), any time effect which is common to the groups compared can be eliminated, and the standard deviation due to random causes—mainly sampling—calculated. This standard deviation is made the basis of any test of the significance of a difference between groups. Otherwise the statistical treatment is similar to that used in our work on carbohydrate transport.

(c) *Preparation of Material.*

This followed the lines already described (9). Bark and wood were separated, and, when necessary, the fresh weights determined. The material was then cut into small fragments, and samples taken for drying in a steam oven. The bulk was placed in freezing-tubes and frozen for twenty-four hours in a salt-ice mixture at approximately -15° C. It was removed from the freezing-tube in small portions and at once pressed in a vice. The expressed sap was filtered through fine lawn, collected in a test-tube jacketed in an ice-salt mixture, and kept frozen in this tube until the time came to take samples for analysis. It was then thawed, thoroughly mixed, and kept in an ice-water jacket while samples for analysis were taken. Filtering through the lawn was rapid, consequently the sap remained at room temperature for a short time only.

The determinations made on the expressed sap were, reducing sugars, total sugars after inversion with invertase, total crystalloid N, amide N, free amino N, nitrate N, and ammonia N; the weight of water contained in 5 cc. of sap was also determined. This allows the results to be expressed as concentrations per 100 grm. water. The hydrogen-ion concentration was also determined in some experiments.

As nitrogen has not hitherto, so far as we can ascertain, been estimated on the sap expressed from frozen material, a comparison was made of the concentration of crystalloid N in thirty-four samples; half of each sample was frozen before expression, while from the other half the sap was expressed immediately on collection. The mean concentration for the frozen material

was 138·7 mg. per 100 grm. water, and for the fresh material 122·5 mg., a difference of about 11 per cent. in favour of the frozen material. The difference is small and is doubtless due to the effect of freezing on the permeability of the membranes (cf. Dixon and Atkins (5)). Apart from the small percentage difference in level, the agreement between the fresh and frozen material is extremely good. The correlation coefficient is +·9832, and the regression equation is

Concentration in frozen sap = (1·124 × Concentration in fresh sap) + 1·1 mg.

There is therefore no reason to suppose that the small and inevitable differences in the time taken to freeze different samples cause any disturbance in the protein-crystalloid N relations.

(d) *Methods of Analysis.*

1. *Estimation of Moisture and Carbohydrates.* The methods used were similar to those employed in our work on the Transport of Carbohydrates.

2. *Hydrogen-ion Concentrations* were determined with the quinhydrone electrode.

3. *Estimation of Nitrogen.*

(1) *Total N* was determined on the dried powder by Ranker's (11) modification of the Kjeldahl method. This includes nitrate N.

(2) *Crystalloid N* (non-protein N) was determined on the sap, after removal of protein with Merck's colloidal ferric hydroxide (cf. Thomas (12)). Three lots, each of 10 c.c. of sap, were measured out and cleared with ferric hydroxide. The filtrates were made acid to litmus with acetic acid and evaporated to dryness *in vacuo*. The nitrogen present was then determined as for total N.

(3) *Amide N* was determined usually on a 25 c.c. sample of the sap after removal of the proteins. The filtrate was adjusted to 200 c.c. and boiled for 3 hours with 12 c.c. of concentrated HCl under a reflux condenser. After neutralization with NaOH, and the addition of 50 c.c. 10 per cent. MgO, the total ammonia was found by distillation for 2 hours *in vacuo*, and from this the free ammonia, as estimated below, was deducted.

(4) *Ammonia N* was estimated on duplicate samples of 25 c.c. of sap after clearing; the free ammonia was found by distillation *in vacuo* as described above. Any volatile bases other than ammonia are of course included. The results serve as a blank for amide N.

(5) *Nitrate N* was determined on the duplicate residues from the ammonia estimation. Both were made up to 200 c.c. and treated with 5 c.c. of alcohol and 15 c.c. of 40 per cent. NaOH. To one was added 1 grm. Devarda alloy. After fitting to the condenser, the two flasks were left

for two hours before distillation of the ammonia. The difference between the ammonia from the two flasks was taken as that due to reduction of nitric N; allowance, of course, being made for the nitrogen from the Devarda blank. Burrel and Philips (1) found a loss of nitrate N by this method in the presence of amide N. To test the extent of this loss, we added 20 mg. of nitrate N to 25 c.c. of bark sap. As the recovery was only about 95 per cent., the results for nitrate N will be on the low side.

(6) *Free-amino N.* 10 c.c. samples of sap were taken, protein was removed with colloidal ferric hydroxide, and colouring matters, which might cause frothing in the Van Slyke (14) apparatus, were removed by boiling with 1 grm. carbon. No loss of glycine added to the sap, could be detected as a result of adsorption by the carbon. The filtrate was made up to 50 c.c. and free amino N determined in the micro Van Slyke apparatus. This determination includes not only the nitrogen of amino-acids but also the nitrogen of the amino group of asparagine and of ammonia. The results for the latter will be on the low side.

As in the present study we are concerned with gradients and movement of definite chemical substances and not with the amounts of amide N and amino N, the results are expressed as follows:—

Asparagine N. The assumption has been made that all the amide N present is in the form of amides of the asparagine type. The asparagine N is therefore taken as twice the amide N.

Amino-acid N is found by deducting ammonia N and the amino N due to asparagine from the total amino N recorded by the Van Slyke method. We will refer to this fraction as amino-acid N.

Residual N represents the crystalloid N not accounted for by the sum of the asparagine N, the amino-acid N, the nitrate N, and the ammonia N. No attempt has been made to ascertain the nature of the substances included.

Protein N is found by deducting crystalloid N (non-protein) from the total N.

It will be evident that the value calculated for residual N depends on the completeness with which the fractions are recovered by the Kjeldahl method on the one hand and the special methods for the fractions on the other. We therefore added known amounts of glycine, asparagine, and sodium nitrate to bark sap before clearing, and determined the percentage recovered. The recovery by the Kjeldahl method was never less than 98 per cent., even in the case of nitrates; the recovery of the nitrogen of glycine by the Van Slyke method averaged 102·0 per cent. (two determinations), the recovery of the amino group of asparagine by the same method averaged 101·7 per cent. (three determinations); the recovery of amide N was 96·5 per cent. (seven determinations), and as already indicated the

recovery of nitrate N was only 94·6 per cent. (five determinations) by the Devarda reduction method.

In so far as these figures are a guide to what happens in the analysis of a sap, about 2·6 per cent. of the asparagine N might be recorded as amino-acid N. The sum of the amino N and the amide N, however, would be very nearly correct, 99 per cent. recovery, so that the residual N would only be increased by about 5 per cent. of the nitrate N figure. This is relatively a very small correction.

Since the attempt to study the distribution of amino-acids as distinct from acid amides in the sap has not hitherto, so far as we are aware, been made, some further comment is desirable. It appears to be generally assumed that the amide N obtained on mild hydrolysis is almost entirely due to acid amides of the asparagine type. Chibnall (4) concludes, from experiments in which he measured the recovery of crystalline asparagine, that not more than 60 to 70 per cent. of the amide N of the leaf sap can safely be ascribed to asparagine; but that a large part of the remainder is probably derived from asparagine, and may exist in the form of di- or poly-peptides. He recognizes that proteose might yield amide N on mild hydrolysis, but, since he obtained no amide N at night, when proteoses were still present in quantities as great as during the day, he concludes that the contribution of proteoses to amide N is not appreciable.

Now if the amide N is entirely due to acid amides of the asparagine type, then the amount of free amino N must be at least equal to the amide N. In Chibnall's experiments, and in our experiments, on the leaf there is always an excess of amino N, which we take to indicate free amino-acids. The same is true, with very few exceptions, for the stem tissues in our experiments. In one experiment, however, in which the bark was allowed to lose water by exposure on the bench, the free amino N was definitely less than the amide N, giving a negative figure for our estimate of amino-acid N. In the carpel wall of the young bolls a similar deficit of amino N was observed. The deficits were in all cases relatively small, amounting at most to 17 per cent. of the amide N figure, but the general run of the figures suggested that the deficits could not be due to analytical errors. The same phenomenon occurs moreover, even more markedly in the results of other workers.

Thus, in analyses by Thomas (13) of the one year branch growth of two trees of Pyrus Malus, a deficit in amino N is shown by one tree on seven occasions out of ten during the season, and by the other tree on four occasions out of nine. The deficit ranges from 2 per cent. to 69 per cent. of the amide N, and even when we take all the observations, including those where amino N is in excess, there is an average deficit for both trees of about 10 per cent. Another example is the analysis by Jodidi and Wangler (7) of amide and amino N in cereal grain. Out of three rye, four wheat,

and three oat varieties examined, one rye, two wheat, and one oat variety show a deficit in amino N, the deficit ranging from 7 to 21 per cent. of the amide N. In these cases, however, amino N was estimated by the formol titration method, which in our experience with cotton plants does not recover as much amino N as the Van Slyke method. In the cereal grain Jodidi and Wangler note the presence of a considerable quantity of polypeptides. It seems possible that these might yield some amide N on mild hydrolysis, but very little amino N in the direct amino N estimation. The possibility cannot, however, be tested on the data presented.

It seems probable therefore, that there may be at times in the sap nitrogen compounds which, like asparagine, yield amide N on mild hydrolysis, but which, unlike asparagine, do not yield an equivalent amount of amino N in the direct Van Slyke determination. Subtraction from the total amino N, of an amount equal to the amide N must give, therefore, a minimal value for amino-acid N, and the estimates for amino-acids must be interpreted with this possibility in mind. It might be argued that the correct procedure in the circumstances is to discuss amide N and amino N rather than asparagine (= acid amide) N and amino-acid N. The interest of estimating both amide N and amino N lies, however, in the possibility of their showing a difference in behaviour, and this difference in behaviour is most readily exhibited by considering, on the one hand, amide N, and on the other, the difference between amino N and amide N. In our own observations we find striking and consistent differences in distribution between acid amide N and amino-acid N.

(e) *Expression of Results.*

Concentrations are expressed per 100 grm. water. This method has the advantage of being independent of temperature fluctuations, which affect volume concentrations, and also, that insoluble or colloid substances, determined on the dried material, can be expressed on the same basis and their amounts relative to the soluble or crystalloid fractions exhibited. The concentrations of nitrogenous substances are, unless otherwise stated, expressed as the number of mg. N per 100 grm. water, and the concentrations of sugars as the number of grm. per 100 grm. water.

When changes during a relatively short time interval in the absolute amount of any substance present in any mature organ or region of the plant are under investigation, the results are presented on the basis of the mean residual dry weight.[1] This applies particularly to short period experiments on transport or on diurnal variation. In experiments of longer duration or experiments on rapidly growing organs such as bolls, or in general in any case where there is evidence that growth has taken place, the data are

[1] Residual dry weight = Dry weight less total carbohydrates (cf. 9).

presented on the per sample basis (i. e. the amounts given are those calculated as being present in the actual samples as collected). Evidence of growth is furnished by an upward trend in the residual dry weight.

Section 3. Vertical Gradients in the Plant.

(a) *Vertical Gradients in the Bark* (*Experiment* 1).

(1) *Procedure.* The plants, which were eleven and a half weeks old and had a mean height of 90·7 cm., were without flowers or bolls, as the flower-buds had been periodically removed. Samples were collected at 5 p.m. on September 26 and at 5.30 a.m. on September 27, 1927. Two samples each of 100 plants were taken at each collection. Two regions of stem were sampled: the Upper consisted of a section of 35 cm. immediately above the third fruiting-branch, while the Lower consisted of a similar length immediately below the third fruiting-branch. Leaves had been removed from the stem below the first fruiting-branch 10 days before the observations began. The Upper region was thus wholly in the foliage region, while the major part of the Lower region lay between the foliage region and the roots.

The bark was stripped from the wood and frozen. The following determinations were made on the sap expressed from it; crystalloid N, asparagine N, nitrate N, ammonia N, reducing sugars, and sucrose. The water in 5 c.c. of sap was not determined. The results are therefore expressed as volume concentrations. No analyses were made on the dried bark or on the wood. The weather was bright and dry.

(2) *Results.* Table I shows the mean concentrations for the two samples taken at each collection. In view of the small number of paired samples available for calculation of the standard deviation, and of the great difference in concentration between the Upper and Lower regions of the bark, the results for the two regions have been pooled and the standard deviation calculated as a percentage of the mean for duplicate samples.

Before discussing the vertical gradients it will be of interest to note the changes that occurred in the concentrations of sugar and crystalloid N during the night.

It will be seen that there was a considerable fall in the concentrations of sucrose and of reducing sugars, and that the fall is more marked in the Upper region, which was nearer to the leaves. Organic crystalloid N also shows a fall in both regions; in the Upper region this is mainly due to a drop in the concentration of asparagine, but in the Lower region it is almost accounted for by the amino-acid + residual N fraction. On the whole there does appear to be a significant drop in concentration of the organic crystalloid N during the night, but this change may, in part, be due to an increase in moisture content. The very definite diurnal changes in sugar concentration are, however, far greater than could be accounted for by

moisture changes and stand in sharp contrast to the small and doubtful changes in the crystalloid nitrogen fractions.

TABLE I.

Concentrations in the Bark Sap.

(Mg. nitrogen or grm. sugar per 100 c.c. of Sap.)

	Upper Region.		Lower Region.		Standard Deviation.
	5.0 p.m.	5.30 a.m.	5.0 p.m.	5.30 a.m.	Per cent.
Total Cryst. N	105·9	101·7	219·4	204·6	3·17
Organic Cryst. N	95·3	85·7	192·4	180·8	3·48
Asparagine N	54·1	46·0	141·3	140·4	4·23
Amino-acid + Residual N	41·2	39·7	51·1	40·4	7·17
Nitrate N	9·4	12·5	25·8	21·7	11·07
Ammonia N	1·2	3·5	1·2	2·1	—
Total Sugars	5·878	4·645	4·171	3·359	4·03
Sucrose	3·732	3·073	2·778	2·222	4·3
Reducing Sugars	2·146	1·572	1·393	1·137	5·05

Significant Differences. P 0·05 Sig. Diff. = Standard Deviation. × 3·926.
P 0·10 „ „ „ × 3·015.

The most striking difference between sugars and nitrogen compounds lies, however, in the vertical gradients. These are set out in Table II. Following the convention adopted in our work on carbohydrate transport, a positive sign is used where the concentration is greater in the Upper region, and a negative sign where the reverse obtains.

TABLE II.

Vertical Concentration Gradients in the Bark.

(Mg. nitrogen or grm. sugar per 100 c.c. of Sap.)

	5.0 p.m.	5.30 a.m.	Mean.	Standard Deviation of Gradient	Standard Deviation of Mean Gradient.
Total Cryst. N	−113·5	−102·9	−108·2	7·54	5·33
Organic Cryst. N	−97·1	−95·1	−96·1	7·48	5·29
Asparagine N	−87·2	−94·4	−90·8	7·64	5·40
Amino-acid + Residual N	−9·9	−0·7	−5·3	4·30	3·04
Nitrate N	−16·4	−9·2	−12·8	3·09	2·18
Ammonia N	—	+1·4	+0·7	—	—
Total Sugars	+1·707	+1·286	+1·497	0·257	0·182
Sucrose	+0·954	+0·851	+0·903	0·179	0·127
Reducing Sugars	+0·753	+0·435	+0·594	0·111	0·078

It will be seen that while the sugar gradients are, as usual, well marked and positive, the gradients for all the nitrogen fractions, with the possible exception of ammonia, are negative. The major part of the gradient in crystalloid N is due to asparagine; of the other fractions only nitrate N shows any significant difference in concentration between the Upper and Lower regions. The nitrogen gradients change somewhat during the night,

but the changes are relatively small and may well have been due to sampling. On the other hand, the diminution in the sugar gradient during the night appears to be significant.

Now if the movement of organic nitrogen towards the roots takes place in the form of some particular compound, or group of compounds, it might on *a priori* grounds be expected that the mobile form would at least be crystalloid in nature. A number of investigators (e.g. 2, 15) have, in fact, concluded that asparagine is the mobile form, and asparagine does, it will be seen (Table I), constitute the greater part of the crystalloid N in the bark. Yet while sugars, which are the mobile form for carbohydrate transport, exhibit a marked positive gradient, organic crystalloid N compounds, and in particular asparagine, show a very large negative gradient. We have already shown (8) that there is normally, in the main axis below the foliage region, a downward movement of organic nitrogen as well as of carbohydrate towards the roots. Unless this experiment is an exception to that general rule, downward movement of organic nitrogen is here proceeding against a marked concentration gradient of organic crystalloid N, mainly asparagine. A number of possible explanations present themselves, but before testing them, it was considered of first importance to establish whether, in plants showing downward movement of nitrogen, the gradient conditions were similar to those in this case, in which the existence of downward movement is only *inferred* but not demonstrated. The next experiment was, therefore, a ringing experiment of the type already described in our carbohydrate work (9). In such an experiment the normal or unringed plant provides data on the vertical gradients, while a comparison of the total amounts of nitrogen found in the stem above and below the *ring*, with those found in the corresponding regions of the stem of the normal plant, provides evidence of the amount and direction of movement.

(b) *The Downward Movement of Nitrogen and Vertical Gradients in Bark and Wood (Experiment 2).*

(1) *Procedure.* The plants had been sown at the same time as those used for Experiment 1, and were as usual without flowers or bolls. The stem below the first fruiting-branch was kept bared of leaves and branches. At 5.30 a.m. on October 6, 1927, half the plants were ringed immediately below the first fruiting-branch. The time table will show the sequence of events.

October 6. 5.30 a.m.	Ringing of half the plants and Initial collection of Normal or Unringed Plants.
October 6, 11.0 a.m. „ „ 4.30 p.m. „ 7, 5.30 a.m.	3 Collections of Ringed and Normal Groups.

For each group there were two samples per collection, each of 40

plants. Two regions of the stem were sampled. The Upper region consisted of a 20 cm. section of stem above the first fruiting-branch (i.e. above the ring in the Ringed group) and the Lower region of a similar section below the first fruiting-branch. In the laboratory the stem samples were trimmed to 18 cm., the portions abutting on the first fruiting-branch being rejected in both groups. Bark and wood were separated, and crystalloid N and sugars determined on the expressed sap. Total N and reserve carbohydrates were determined on the dried material. The weather was bright and no rain fell during the period.

(2) *Results.* We will consider first of all the question of movement. The mean weights of nitrogen and of carbohydrates in the Upper and Lower regions of the stem of the Ringed and Normal groups are shown in Table III. The initial collection is not, of course, included. The results are expressed on the basis of the mean residual dry weight.

It will be seen that in the Upper region both carbohydrate and nitrogen have accumulated in the Ringed group in excess of the normal amount. In the Lower region the reverse obtains. It may thus be taken as established that there is a downward movement of carbohydrate and nitrogen from the leaves towards the roots in the plants with which we are dealing.

TABLE III.

Grm. Total Carbohydrates and Mg. Nitrogen per 18 cm. of Stem (Bark + Wood).

	Total Carbohydrates.			Nitrogen.		
	Normal.	Ringed.	Significant Difference.	Normal.	Ringed.	Significant Difference
Upper Region	1·675	1·858	0·068	95·63	100·53	4·78
Lower Region	2·255	1·900	0·079	119·07	110·45	4·84

TABLE IV.

Mean Concentration in Upper and Lower Regions of Bark and Wood of Normal Plants.

	Bark.			Wood.		
	Upper Region.	Lower Region.	Significant Difference.	Upper Region.	Lower Region.	Significant Difference.
Protein N . .	292	296	24·9	206	220	32·6
Total Cryst. N .	146	200	15·9	186	156	13·2
Total Sugars .	5·635	4·543	0·313	1·127	1·003	0·093

The mean concentrations of protein N, crystalloid N and total sugars in the Upper and Lower regions of the Normal group are recorded in Table IV. The significant difference (for P = ·05) between the concentrations in the two regions is also shown. In the bark total sugars show the usual well-marked positive gradient. The average difference in concentration between

the two regions is 1·09 per cent. sugar, while the significant difference is only 0·31 per cent. The gradient in crystalloid N is again definitely negative; the average difference in concentration between the two regions is 54 mg. and the significant difference is only 15·9 mg. It is clear, therefore, that the downward movement of nitrogen in the bark proceeds against a considerable gradient of crystalloid N. The concentration of protein N, on the other hand, is only slightly greater in the Lower than in the Upper region. This small gradient is not statistically significant. Thus while the sugar gradient in the bark is well marked and positive, the gradients in nitrogen compounds are either negligible (protein N) or markedly negative (crystalloid N).

The case is quite different in the wood. Here the sugar gradient, although positive and statistically significant (mean value 0·124; significant level 0·093) is very small, while there is a considerable positive gradient in crystalloid N, the average value being 30 mg. This gradient is quite well established for the significant level is only 13·2 mg.

In view of the rapid interchange that occurs between wood and bark this lack of correspondence between the direction of the nitrogen gradients is very striking. We noted in Part I (8) that, when transport is interrupted by ringing the bark, both nitrogen and carbohydrates accumulate in the bark and wood above the ring, while there is depletion in both tissues below the ring. In the experiments discussed in that paper it was found that the change in nitrogen content resulting from ringing was greater in the wood than in the bark. For carbohydrates, on the other hand, the response was greater in the bark than in the wood.[1] The difference in the direction of the vertical nitrogen gradients in bark and wood cannot therefore be connected with any isolation of wood from bark. The question consequently arises whether the positive gradient of crystalloid N in the wood may not indicate a similar gradient in the bark of some mobile form of nitrogen, with which the mobile nitrogen in the wood is in equilibrium. In the absence of any analysis of the composition of the crystalloid N of bark and wood in this experiment, the suggestion cannot be discussed further at this stage. As it does, however, appear to afford a possible clue to the interpretation of the phenomena observed, it was decided to obtain further data on the vertical gradients in bark and in wood, and in addition to see whether the concentrations in the leaves bore any relation to their vertical position on the stem.

(c) *Vertical Gradients in Leaf, Bark, and Wood (Experiment 8).*

(1) *Procedure.* The plants employed in this experiment were not bearing flowers or bolls and had a mean height of 142 cm. The regions of

[1] In *this* experiment the major part of the response (roughly 66 per cent.) both in nitrogen and in carbohydrates occurs in the bark, but the response in the wood is quite definite and fully significant.

stem sampled were as follows: Upper region—24 cm. immediately below the middle fruiting-branch; Lower region—18 cm. immediately below the first (lowest) fruiting-branch. There was an intermediate region between the bottom of the Upper region and the top of the Lower region: this region, which had on the average a length of 30·4 cm., was not sampled. The mean distance between the mid-points of the two regions sampled was thus 51·4 cm. The Upper region was thus situated at about the middle of the foliage region, while the Lower was just below it. Samples of leaves were taken from the fruiting-branches just above the Upper and just above the Lower regions. The stem below the first fruiting-branch bore no leaves.

Two samples were taken, one at 9.40 a.m., the second at 12.40 p.m. on January 3, 1928. There were seventy-two plants per sample and from each plant, in addition to the stem samples, four leaves were taken, i.e. two from each of the foliage regions sampled. The weather was dry.

The determinations made on the material were:—total N, crystalloid N, asparagine N, nitrate N, ammonia N, sucrose, and reducing sugars. Amino N was not determined, consequently only the sum of the two fractions, amino-acid N and residual N can be given.

(2) *Results.* The bark was separated into two zones, inner and outer, by the subdivision method previously described (10). The inner and outer fractions were analyzed separately. As, however, we are concerned at the moment with gradients in the bark as a whole, we have calculated the concentrations in the whole bark, by summing for each compound the weights found in the two fractions, and dividing by the combined weight of water, i. e. by taking the weighted mean of the concentrations in the two fractions. The concentrations in the inner and outer fractions will be considered later.

The mean concentrations in leaf, bark, and wood, for the two samples from Upper and from Lower regions are recorded in Table V A. Since only two samples were taken from each region, and these at different times, calculation of a standard deviation for the vertical gradients is not possible. The gradients (Table V B) are therefore given separately for each sample.

It will be seen that conditions in the bark are similar to those recorded previously; there is the usual positive gradient in sugars, due mainly to sucrose, and a considerable negative gradient in organic crystalloid N, due almost entirely to asparagine. The gradient in nitrate N is positive instead of negative as before (Experiment 1) but the large variation between the two samples suggests that the gradient is not significant. There would appear to be a definite, if small, positive gradient in ammonia N, but no significant gradient in the other nitrogen fractions.

As in the last experiment the nitrogen gradients in the wood are, on the whole, in the opposite direction to those in the bark. The small gradient in total sugars is negative (sucrose and reducing sugars show gradients in opposite directions), while there is a definite positive gradient

in total crystalloid N. The organic crystalloid N shows a fairly consistent positive gradient, 11·6 and 14·3, but there is considerable variation in the individual gradients for the two fractions, i.e. asparagine N and amino-acid + residual N, into which this is subdivided.

TABLE V.

A. *Concentrations.*

(Mean of the two Samples at 9.40 a.m. and 12.40 p.m.)

	Leaf.		Bark.		Wood.	
	Upper.	Lower.	Upper.	Lower.	Upper.	Lower.
Total N	1529·0	1274·0	556·4	634·2	403·4	382·4
Protein N	1359·0	1124·0	351·2	354·8	263·7	270·0
Total Cryst. N	170·2	149·7	205·2	279·4	139·7	112·4
Organic Cryst. N	152·4	132·6	184·6	264·2	110·7	97·8
Asparagine N	13·4	7·2	132·8	211·2	38·5	31·8
Amino-acid + Residual N	139·0	125·4	51·8	53·0	72·2	66·0
Nitrate N	9·0	8·3	16·4	11·9	25·3	11·8
Ammonia N	8·8	8·8	4·2	3·3	3·7	2·8
Total Sugars	3·253	2·593	5·661	4·886	1·697	1·767
Sucrose	0·199	0·346	2·571	2·058	0·888	1·154
Reducing Sugars	3·054	2·257	3·090	2·828	0·809	0·613

B. *Gradients.*

	Leaf.		Bark.		Wood.	
	9.40 a.m.	12.40 p.m.	9.40 a.m.	12·40 p.m.	9.40 a.m.	12.40 p.m.
	a.	b.	a.	b.	a.	b.
Total N	+238·1	+272·8	−64·6	−91·0	+11·1	+31·0
Protein N	+217·6	+252·4	+18·8	−26·1	−15·6	+3·0
Total Cryst. N	+20·5	+20·4	−83·4	−64·9	+26·7	+28·0
Organic Cryst. N	+23·2	+16·4	−92·3	−66·9	+11·6	+14·3
Asparagine N	+11·8	+0·6	−87·7	−69·1	+10·2	+3·2
Amino-acid + Residual N	+11·4	+15·8	−4·6	+2·2	+1·4	+11·1
Nitrate N	—	+1·4	+8·1	+1·0	+14·7	+12·4
Ammonia N	−2·7	+2·7	+0·8	+1·0	+0·4	+1·40
Total sugars	+0·667	+0·655	+0·846	+0·704	−0·075	−0·065
Sucrose	−0·111	−0·182	+0·568	+0·457	−0·253	−0·279
Reducing Sugars	+0·778	−0·837	+0·278	+0·247	−0·178	+0·214

The vertical gradients in the leaf are particularly interesting. As in the bark, the total sugars show a marked positive gradient. The negative gradient in crystalloid N found in the bark is, however, not paralleled in the leaf. The gradient here is positive, as in the wood. This gradient is mainly due to the amino-acid + residual N fraction, though there is also a positive asparagine gradient in the first sample; the other crystalloid fractions do not show any well-defined gradients. The most striking vertical gradient in the leaves is, however, the positive gradient in protein N. In

this respect the leaves differ from both bark and wood, for neither of these show any consistent vertical gradients in protein N.

It will be seen that, in the two tissues which we may suppose to be in dynamic equilibrium with the bark—the leaves as supplying organic nitrogen to the bark, and the wood as supplying or receiving from the bark, according to circumstances—there is a positive gradient in organic crystalloid N. Can we infer from these gradients in leaves and wood a gradient of some mobile form of nitrogen, in the same direction, in the bark? It will be remembered that in the previous experiment we found in the wood a positive gradient of crystalloid N. No fractionation of the crystalloid N was attempted in that experiment, and it was conceivable, therefore, that the positive gradient might be due entirely to inorganic nitrogen. In that case the inference outlined above would have been very doubtful. It will be seen from this experiment, however, that although rather more than half the positive gradient in crystalloid N in the wood is due to inorganic nitrogen there still is a positive gradient in organic crystalloid N. The presence of a similar gradient of about the same order in the leaves strengthens the inference. As to the fractions of which the gradient in organic crystalloid N in leaf and wood is composed the case is not as clear. In the leaves the gradient is mainly due to amino-acid + residual N, and the gradient in this fraction is fairly consistent as between the two samples, but there is also an asparagine N gradient in the first sample. In the wood the gradient is due both to asparagine N and amino-acid + residual N, but their relative importance is reversed in the two samples.

To sum up, though the presence of positive gradients of organic crystalloid N in the leaves and in the wood, suggests that there may be a positive gradient of some mobile form of nitrogen in the bark, consideration of the fractions composing the gradients makes the evidence somewhat conflicting. The gradients in the leaf suggest amino-acid + residual N, while those in the wood suggest either amino-acid + residual N or asparagine N. Argument from the leaf gradient is perhaps the stronger since we may reasonably assume during the experiment an export of organic nitrogen from leaf to bark, while we can make no assumptions as to the direction of movement between bark and wood in the two regions of stem sampled.

The data suggest two possibilities: (1) some part of the amino-acid + residual N fraction may be the mobile compound and may show a positive gradient in the bark; (2) the organic crystalloid N in the leaf and wood may be in dynamic equilibrium with organic crystalloid N in the conducting cells of the bark, but the positive gradient in the *conducting cells* may not show up in analyses of the *bark as a whole.*

The second possibility will be considered at a later stage. To test the first we must split up the amino-acid + residual N fraction. Estimation of amino N was, in fact, attempted in two of the experiments so far described

(Experiments 1 and 8), but we were then using the formol method which, as we found subsequently, recovers only about 60 per cent. of the amino N of the bark sap. This was accordingly discarded and the Van Slyke method adopted.

As data, obtained by the Van Slyke method, for amino N are available from the initial collection of an experiment on Reversal of Movement in the bark, carried out on January 16, 1928, we will consider the relevant data from that experiment next.

(d) *Further Observations on Vertical Gradients (Experiment 9).*

(1) *Procedure.* The samples of the Upper region consisted of the stem between the 11th and 17th nodes from the apex. The Lower region extended over six internodes counting downwards from the base of the Upper region, and terminated at the first fruiting-branch, below which there were no leaves or branches. As usual the plants were without bolls or flowers. The mean distance between the mid-points of the Upper and Lower regions was 27·33 cm. Two samples, each of 54 plants, were taken at 9.0 a.m. on January 16, 1928. The determinations made were: total crystalloid N, asparagine N, amino-acid N, ammonia N, sucrose, and reducing sugars. Owing to insufficient sap it was not possible to determine nitrate N. Total N and reserve carbohydrates were determined on the dried material. As in the preceding experiment the bark was subdivided into inner and outer zones. The results for the separate zones will be considered in the next section.

(2) *Results.* Table VI shows the mean concentrations and gradients in the whole bark.

TABLE VI.

Concentrations and Gradients between Upper and Lower Regions of Bark.

	Whole Bark.		
	Concentrations.		Gradients.
	Upper Region.	Lower Region.	
Total N	448·4	483·9	−35·5
Protein N	328·7	324·4	+4·3
Total Cryst. N	119·7	159·5	−39·8
Asparagine N	62·19	100·22	−38·03
Free Amino N	18·18	23·58	−5·40
Residual N + Nitrate N	38·43	34·12	+4·31
Ammonia N	0·90	1·62	−0·72
Total Sugars	6·415	5·852	+0·563
Sucrose	3·296	2·787	+0·509
Reducing Sugars	3·119	3·065	+0·054

Total crystalloid N and asparagine show gradients similar to those recorded previously. The chief interest lies in the results for the amino-acid fraction. This resembles asparagine in its vertical distribution, though the relative difference between Upper and Lower regions is much smaller. The negative gradient, −5·4 mg. per cent., is quite definite, for the significant difference between Upper and Lower regions is only 3·8 mg. per cent. The existence of a negative gradient in amino-acid N makes it probable that there is a positive gradient in residual N, for the sum of these two fractions has been found previously to give either a positive gradient or only a small negative gradient. In this experiment residual N + nitrate N show a small positive gradient, but the absence of data for nitrate N makes it impossible to test the possibility further.

In general it may be said that the negative gradients in asparagine and amino-acids combined are more than sufficient to account for the negative gradient in total organic crystalloid N. The question arises therefore whether these nitrogen fractions may not be temporary storage compounds, in which case the negative gradient in crystalloid N would be a phenomenon mainly of storage and bear no direct relation to transport. If there is, in the bark as a whole, a concentration gradient of the mobile form in the direction of transport, then the most likely mobile form would seem to be residual N.

As yet we have considered only the concentration gradients in the bark as a whole. We referred earlier to the possibility that a positive gradient of mobile nitrogen in the conducting cells might not show up in analyses of the bark as a whole. Assuming, as is very probable, that longitudinal movement is limited to the sieve-tubes, it will be evident that a positive gradient of organic crystalloid N in the sieve-tubes might, as a result of greater storage in the rays and cortex of the Lower region of bark, be masked by the negative gradient [1] in these tissues. If this were the case then the inner zone of the bark, in which the sieve-tubes are mainly located, might show either a positive gradient or at least a negative gradient less steep than that in the outer zone.

It should be noted that, while the theory of positive gradients in the conducting channels, masked by negative gradients in the storage tissues, may explain the direction of movement in the conducting channels, the problem of movement against an apparent gradient is thereby only pushed one stage further back; for, in the Lower region of the stem, movement from the conducting channel into the neighbouring tissues must then take place against a very steep gradient. Also when, in a lower region of stem below a ring, there is a depletion of the nitrogen in the wood, movement of nitrogen

[1] The negative gradient in the storage tissues (rays and cortex) would not need to be very steep, since these tissues combined have about four times the volume of the sieve-tube tissues.

from wood to bark must take place against a very steep gradient between the rays of the bark and the wood. *A priori* however, it does not seem unreasonable to locate the phenomenon of movement against an apparent gradient at the junction of two different tissues rather than within the chain of cells of similar structure which form the conducting channels. It is, in any case, a possibility which must be proved or disproved before further progress with the problem can be made.

(e) *Vertical Gradients in Inner and Outer Halves of Bark (Experiments 8 and 9 continued).*

(1) *Procedure.* The bark in Experiment 9, which we have just considered, was divided into an outer and inner fraction. As in previous experiments (10) on radial subdivision of the bark, we take the fresh weight of each fraction, relative to the total fresh weight of the bark, as an estimate of its relative cross sectional area. In this experiment the inner fraction was on the average 44·9 per cent. and the outer 55·1 per cent. of the total, but naturally the percentage weights varied somewhat with different samples. The standard deviation for a sample was 2·8 per cent. It was considered advisable, therefore, to calculate the theoretical concentrations in inner and outer *halves* on the simple assumption that the concentration at the junction of the two fractions is the mean of the two concentrations observed, and that the concentration changes linearly from that point in either direction. A diagram will make the method of calculation clear, though actually arithmetic methods were employed.

Figure 1 shows the concentration of total crystalloid N in inner and outer zones of one of the samples of bark from the Lower region of the stem. The concentrations are plotted as histograms, the base line being proportional to the fresh weight of each zone. The line TMT_1 represents the smoothed distribution of concentrations, and is obtained by joining the mid-point M between the two parts of the histogram to the centre of the line at the top of each part of the histogram, and prolonging these straight lines to meet the vertical axes 0·0 and 1·0. The concentrations in inner and outer *halves* are obtained by integrating the areas below the line TMT_1, on either side of the central axis (dotted line). In the case plotted the observed concentrations are 147·8 (inner fraction) and 97·7 (outer fraction): the concentrations calculated for inner and outer *halves* are 144·3 and 94·9 respectively. The correction involved is small but, for the sake of uniformity of treatment, it was thought advisable to treat all cases of subdivision into two zones in this way.

(2) *Results.* The concentrations calculated for the inner and outer halves of the bark are shown in Table VII; the figures given are the means for the two samples taken. The gradients are shown on the right of the Table.

It will be noticed that the negative gradients in asparagine and amino-acid are even more marked in the inner half of the bark than in the outer.

TABLE VII.

Concentrations in Inner and Outer Halves of the Bark and Vertical Gradients (Experiment 9).

	Concentrations.				Vertical Gradients.	
	Inner Half of Bark.		Outer Half of Bark.			
	Upper Region.	Lower Region.	Upper Region.	Lower Region.	Inner Half of Bark.	Outer Half of Bark.
Total N	479·9	504·6	416·7	463·0	−24·7	−46·3
Protein N	333·1	303·4	323·8	345·7	+29·7	−21·9
Total Cryst. N	146·8	201·2	92·9	117·3	−54·4	−24·4
Asparagine N	74·2	123·2	50·1	76·1	−49·7	−26·0
Amino-acid N	22·6	30·0	13·6	17·0	−7·4	−3·4
Residual N + Nitrate N	49·8	45·5	27·6	22·7	+4·3	+4·9
Ammonia N	0·2	1·8	1·6	1·5	−1·6	+0·1

TABLE VIII.

Concentrations in Inner and Outer Halves of the Bark and Vertical Gradients (Experiment 8).

	Concentrations.				Vertical Gradients.	
	Inner Half of Bark.		Outer Half of Bark.			
	Upper Region.	Lower Region.	Upper Region.	Lower Region.	Inner Half of Bark.	Outer Half of Bark.
Total N	582·0	667·5	530·8	600·5	−85·5	−69·7
Protein N	345·7	345·3	356·7	364·6	+0·4	−7·9
Total Cryst. N	236·3	322·2	174·1	235·9	−85·9	−61·8
Total Organic Cryst. N	214·9	305·8	154·2	221·8	−90·9	−67·6
Asparagine N	143·6	231·4	121·9	190·5	−87·8	−68·6
Amino-acid + Residual N	71·3	74·4	32·3	31·3	−3·1	+1·0
Nitrate N	17·6	13·8	15·2	10·0	+3·8	+5·2
Ammonia N	3·8	2·6	4·7	4·1	+1·2	+0·6

The general level of concentration is, of course, higher in the inner half, and the relative difference between the Upper and Lower regions is very much the same in the two halves. There is a small positive gradient in the residual N + nitrate fraction in both halves, but there is clearly no difference between inner and outer gradients. The only masking suggested is in protein N, where a positive gradient of +29·7 in the inner half is masked by a negative gradient of −21·9 in the outer half, so that the positive gradient in the whole bark is only +4·3 (Table VI). The gradients in protein N are, however, small relative to the mean concentration and of

doubtful significance. Substantially the same results are given by the inner and outer halves of the bark in Experiment 8 (Table VIII).

Now on the basis of microscopic drawings of cross sections of the bark, the composition of the inner and outer halves of the bark would be

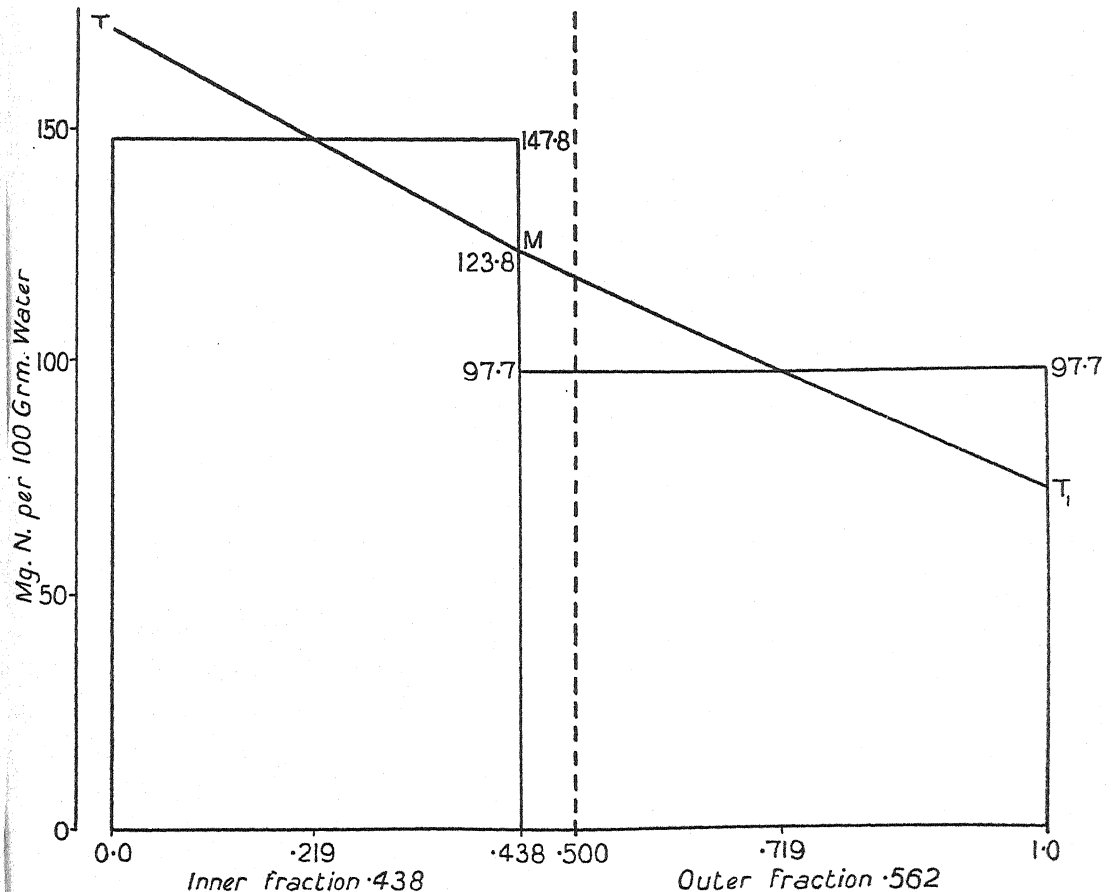


FIG. 1. Showing method of calculating concentrations for inner and outer *halves* of bark.

approximately as follows: inner half, sieve-tubes 30·1 per cent., rays 46·0 per cent., fibres 23·9 per cent.; outer half, sieve-tubes 3·9 per cent., rays 39·4 per cent., fibres 4·4 per cent., cortex 52·3 per cent. It will be seen that the inner half contains nearly all the sieve-tubes, yet this half shows a negative gradient in asparagine N and amino-acid N steeper than that in the outer half.

Similarly the somewhat uncertain evidence in favour of a positive gradient of residual N is no clearer for the inner zone than for the bark as a whole. Thus the evidence is rather against the theory of a positive

gradient, either of asparagine or of amino-acids, in the sieve-tubes which is masked by a negative gradient in the other tissues of the bark. On the other hand the evidence does not exclude such a possibility, for the inner half, although containing most of the sieve-tubes, contains still more ray tissue, and has, moreover, more ray tissue than the outer half. Now if storage of organic crystalloid N in the cortex is very much the same at different vertical levels, while storage in the rays is very much greater in the Lower than the Upper region, then the actual gradient observed in the inner half of the bark might very well be more strongly negative than in the outer half, in spite of a positive gradient in the sieve-tubes. As we shall see later (p. 645) there is some reason for thinking that the rays may differ considerably from the cortex in storage of asparagine. A further possibility is that a positive gradient in the sieve-tubes is masked by a negative gradient in the companion cells. It should be noted finally that although the percentage of sieve-tubes was slightly smaller in the Upper region of the bark than in the Lower, 16·5 per cent. (Upper) as against 17·5 per cent. (Lower), the difference is too small to afford any explanation of the negative gradients in the bark as a whole.

At this stage it may be useful to summarize the data on the vertical crystalloid gradients. The downward movement of nitrogen through the bark takes place against a net gradient of asparagine and amino-acid in the whole bark. The presence of a positive gradient in residual N has not been demonstrated, but may perhaps be inferred. Subdivision of the bark into two halves, while rendering it improbable that there are in the sieve-tubes positive gradients, either in asparagine or amino-acids, which are masked by negative gradients in the other tissues of the bark, does not exclude that possibility. On the whole it would appear that if the downward movement of nitrogen is along a concentration gradient in the bark as a whole, the mobile form is either residual N or some part of the residual N fraction. The presence of positive gradients in leaf and wood is in harmony with such a view.

TABLE IX.

Percentage Composition of Total Crystalloid N in Upper and Lower Regions of Bark.

Fraction.	Percentage of Total Crystalloid Nitrogen.					
	Expt. 1.		Expt. 8.		Expt. 9.	
	Upper.	Lower.	Upper.	Lower.	Upper.	Lower.
Asparagine N.	48·22	66·44	64·69	75·59	51·95	62·81
Amino-acid + Residual N	38·97	21·58	25·23	18·97	—	—
Residual N + Nitrate N	—	—	—	—	32·11	21·39
Amino-acid N	—	—	—	—	15·19	14·78
Nitrate N	10·55	11·20	7·99	4·26	—	—
Ammonia N	2·26	0·78	2·09	1·18	0·75	1·02

Before leaving the subject of vertical gradients in the bark it is of some interest to note (Table IX) that asparagine forms a much greater proportion of the total crystalloid N in the Lower region of the bark. The amino-acid + residual N fraction, on the other hand, is present in much higher proportions in the Upper region. Amino-acids alone (Experiment 9), occupy an intermediate position, the proportion being only slightly greater in the Upper region.

Section 4. Radial Distribution of Concentrations in the Bark.

(a) *Subdivision into Two Zones (Experiments 8 and 9 continued).*

In the two experiments just described (Experiments 8 and 9) the bark was subdivided into two fractions, inner and outer. The concentrations calculated for inner and outer halves of the bark have been recorded in Tables VII and VIII in connexion with the discussion of vertical gradients. It will be seen from these tables that the concentrations in inner and outer halves show striking differences. The radial gradients (inner minus outer) and the relative differences between inner and outer halves are shown in Table X.

Table X.

	Radial Gradients.				Relative Difference[1] (Inner-Outer).			
	Expt. 8.		Expt. 9.		Expt. 8.		Expt. 9.	
	Upper.	Lower.	Upper.	Lower.	Upper.	Lower.	Upper.	Lower.
Total N	+51·2	+67·0	+63·2	+37·6	+9·2	+10·58	+14·1	+7·8
Protein N	−11·0	−19·3	+9·3	−46·3	−3·13	−5·44	+2·8	−14·3
Total Cryst. N	+62·2	+86·3	+53·9	+83·9	+30·4	+30·9	+45·0	+53·1
Asparagine N	+21·7	+40·9	+24·1	+47·8	+16·3	+19·4	+38·8	+47·8
Amino-acid N	—	—	+9·0	+13·0	—	—	+49·7	+55·0
Nitrate N	+2·4	+3·8	—	—	+14·6	+32·0	—	—
Ammonia N	−0·9	−1·5	−1·4	+0·3	−21·2	−44·7	−165·0	+16·6
Amino-acid + Residual N	+39·0	+43·1	—	—	+75·2	+81·5	—	—
Residual N + Nitrate N	—	—	+22·2	+22·8	—	—	+57·0	+66·8

Protein has, on the average, a lower concentration in the inner half, but the difference is relatively small and is not consistent. Total crystalloid N, on the other hand, has in all cases a much higher concentration in the inner half. It will be remembered that the vertical gradients of total crystalloid N were almost entirely due to asparagine; it is interesting therefore to find that, on the average, asparagine accounts for less than half of the radial gradients. The gradient in nitrate N, though positive, is small, while the

[1] In calculating the relative difference, the actual difference between inner and outer halves is expressed as a per cent. of the mean for the two halves.

small gradient in ammonia N is, on the average, negative, so that the positive gradient in the non-asparagine fraction is mainly due to amino-acids and residual N. It will be noted also that the relative change between outer and inner halves is much greater for these fractions than for asparagine.

The radial gradients are very much more steep than the vertical gradients, for the actual concentration differences observed are of the same order, although the radial gradients are measured over a distance of about 1 mm. only, while the vertical gradients are measured over distances of 27 or 50 cm. Vertical and radial gradients in sugar concentration showed a similar behaviour (10).

The interest of the radial gradients in the bark lies in the inference which may be drawn from the data, as to the relative concentrations of different compounds in the different tissues of which the bark is composed. As already noted, nearly all the sieve-tubes are contained within the inner half of the bark. The fact that certain substances have a higher concentration in the inner half may therefore be due to the fact that they exist in the sieve-tubes in higher concentrations than in the other tissues of the bark. This was broadly the explanation given in the earlier paper on carbohydrate transport, of the radial gradients in sucrose and reducing sugars. In that work, however, the bark was subdivided into three zones, and it was found possible, by grouping the data for similarly treated samples, to establish statistically significant correlations between the concentrations of sugar and the distribution of sieve-tubes. On the basis of this statistical analysis estimates were obtained of concentrations in sieve-tubes and bark-parenchyma (ray tissues plus cortex) respectively.

Subdivision of the bark into two zones only does not, of course, give data adequate for such a statistical analysis. Further experiments directed specifically to the study of radial distribution of nitrogen compounds were accordingly carried out in which, as in the previous work on carbohydrates, the bark was subdivided into three zones. Unfortunately, owing to the much greater amount of sap required for the nitrogen determinations, these experiments had to be limited severely in scope. In the carbohydrate work it was possible to obtain, in one experiment, parallel data on radial distribution in both upper and lower regions of the stem of normal plants, of plants with leaves removed, and of plants ringed between the upper and lower regions of stem. In the case of nitrogen only one region of stem has thus far been handled at one time. Nor have more than two treatments been compared at any one time.

In this section an account will be given only of the results obtained on the radial distribution of concentrations in the normal plant. The interest of the data lies in the information given as to the concentrations in the different tissues of the bark. The validity of any inference as to the mobile form of nitrogen will depend on the correctness or otherwise of the assump-

tions made as to the functions of the various tissues. Similarly any inference as to the function of the tissues will depend for its validity on the correct identification of the mobile and storage compounds respectively. For simplicity the results will be discussed on the basis of the usual assumption as to the functions of the three tissue groups, i. e. the sieve-tubes are concerned in rapid longitudinal conduction, the rays mainly in storage and partly in relatively slow radial conduction, and the cortex mainly in storage. Sieve-tubes and companion cells will be treated as a unit, and the fibres will be disregarded.

(b) *Subdivision into Three Zones.*

(1) *Experiment 4.*

In this preliminary experiment the bark was subdivided into three zones in the usual way. The weighted means, calculated from the concentrations found in the three fractions, were compared with the concentrations found in strips of undivided bark. The figures recorded in Table XI represent the means of two samples each of 36 plants.

TABLE XI.

Comparison of Intact and Subdivided Bark.

	Collection 1.		Collection 2.	
	Intact Bark.	Sub-divided Bark.	Intact Bark.	Sub-divided Bark.
Total N . . .	580	599	605	607
Protein N . . .	279	310	321	324
Total Cryst. N . .	301	289	284	283
Sucrose . . .	2·525	2·452	2·760	2·580
Reducing Sugars .	1·435	1·388	1·379	1·392

It will be seen that the differences between intact and subdivided bark are relatively small. There is some loss of sucrose in both collections, while in the first collection the subdivided bark shows more protein N and less crystalloid N than the intact bark. This change, however, is in the same direction as is shown both by the intact and the subdivided bark during the day, and may be due merely to the somewhat longer interval elapsing between collection and freezing in the case of the subdivided bark. On the whole the subdivision has not seriously disturbed the average concentration.

The crystalloid N fractions were not determined in this experiment, and as the radial distribution observed was similar to that in the next experiment, in which the crystalloid fractions were determined, the results will not be discussed in detail. The main feature was that, although the concentration of crystalloid N was much higher in the inner than the outer

zone, the concentration in the middle zone, which is largely composed of ray tissues, was nearly as high in the inner zone, where ray and sieve-tube tissues are present in about equal amount.

(2) *Experiment II.*

(1) *Procedure.* This experiment was carried out as part of an investigation into the factors determining fluctuations in the ratio of crystalloid N to protein N. The experiment will be considered more fully in a later paper, but the data obtained on the Normal or Untreated plants, in the initial collection of samples, may be presented here. In this experiment, carried out on February 14, 1928, the stem samples consisted of 18 inches of stem, between the lowest fruiting-branch and the ground. This region had previously been bared of leaves. There were two samples, each of 46 plants. Each stem was split longitudinally into four quadrants: two quadrants were reserved for further treatment, but the bark on the other two quadrants was at once subdivided into three zones. Each field sample thus provided two sub-samples for the initial observations on radial distribution.

The following determinations were made: total N, total crystalloid N, asparagine N, and amino-acid N, sucrose, and reducing sugars. Nitrate N and ammonia N were not determined. The asparagine figure therefore includes ammonia N.

(2). *Results.* The data are presented in Fig. 2. The base line represents the combined fresh weights of the three fractions. The concentrations for any particular zone are plotted above the mid-point of that part of the base line corresponding to that zone.

The vertical scales used differ for different compounds, but all concentrations plotted in the same graph start from the same (zero) base line. The fresh weights of the three zones are regarded as approximate measures of the cross sectional areas of the zones relative to one another. Fig. 2 shows also the radial distribution of the principal tissues of the bark. Each tissue is expressed as a percentage of the total cross sectional area and the values are the averages for a number of sections. Actually in any one piece of bark the ray tissues would cease abruptly at the line of the starch sheath, and the cortex would start abruptly at the same line. But when a number of samples are taken the distributions overlap. Since the zones of bark analysed are also taken from a large number of different samples, this statistical average distribution of tissues is clearly the appropriate form of distribution.

Table XII, which gives the mean concentrations for all four samples at the mid-points for the three zones, provides a summary of the radial distribution data.

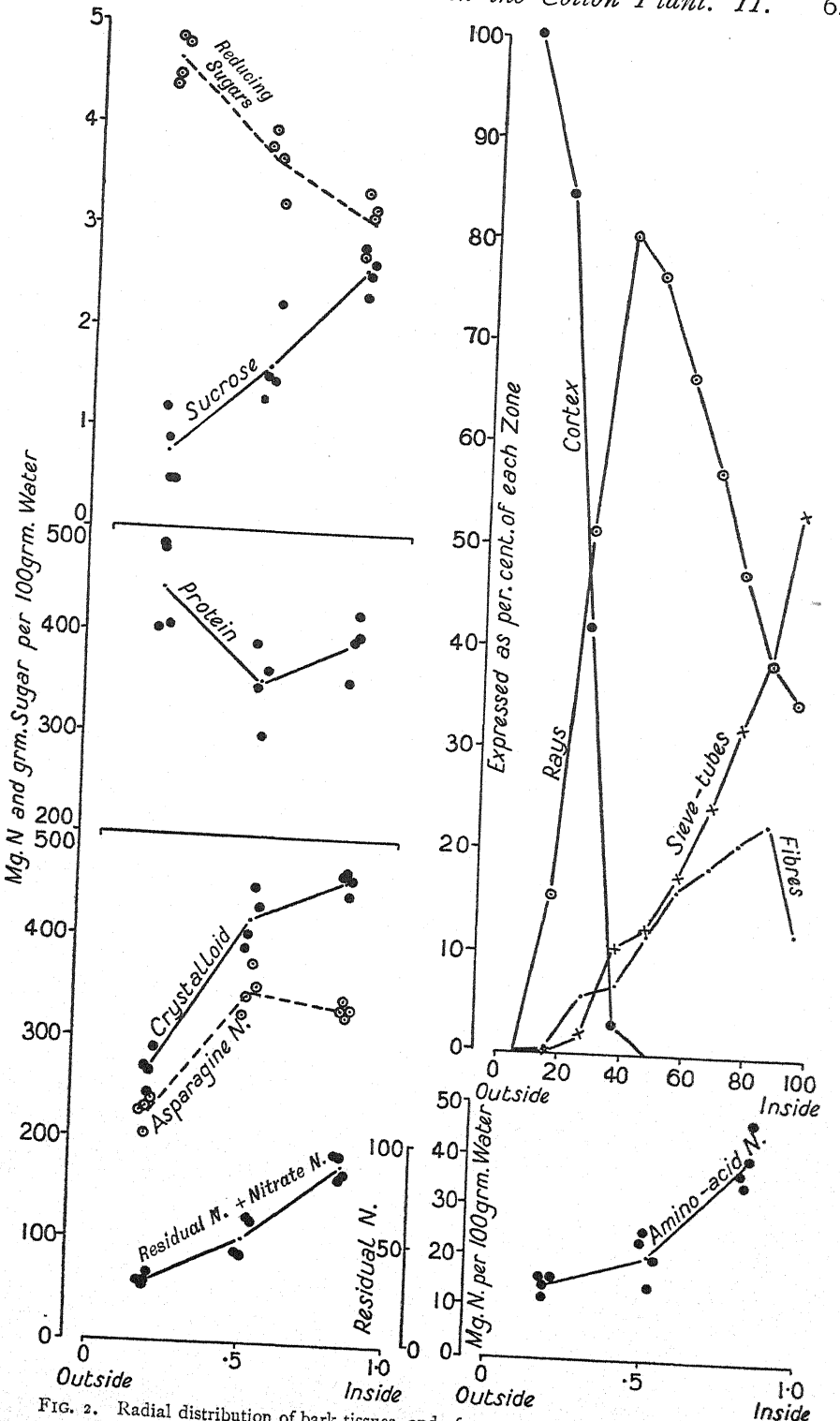


FIG. 2. Radial distribution of bark tissues, and of sugars and crystalloid N fractions.

TABLE XII.

Radial Distribution of Sugars and Nitrogenous Compounds in the Bark.

(Mean concentrations for outer, middle, and inner zones.)

	Outer.	Middle.	Inner.
Total N . . .	708·1	766·6	846·3
Protein N . .	442·5	348·3	390·0
Total Cryst. N .	265·6	418·3	456·3
Asparagine N .	222·9	346·1	329·0
Amino-acid N .	14·2	20·3	39·3
Residual N + Nitrate N .	28·5	51·9	88·0
Sucrose . . .	0·762	1·607	2·565
Reducing Sugars .	4·610	3·651	3·082

It will be seen from the figure that the four samples vary somewhat, as might be expected from the great length of stem taken for analysis. The grouping of the points is, however, good and the general distribution of concentrations across the bark is clear. Sucrose and reducing sugars show gradients of the usual form (10) and it is clear that, as in previous experiments, the distributions of concentrations can very largely be interpreted in terms of the distribution of sieve-tubes. In the case of the nitrogen compounds, however, the only close parallel with the distribution of the sieve-tubes is the distribution of the amino-acid and of the residual N + nitrate N fractions. Asparagine, on the other hand, while it does increase considerably in concentration from outside to inside, shows a marked hump in the middle zone. Now it is in this zone that the ray tissues are most abundant, and it seems at least probable that the concentration of asparagine in the rays may be quite high, possibly higher even than in the sieve-tubes.

In working with sugars it seemed possible to neglect any average difference in concentration between cortex and ray tissues. In the case of nitrogen the simpler classification of the bark tissues into sieve-tube groups and bark parenchyma appears inadequate, and classification must be made into three tissue groups, sieve-tubes, ray tissues and cortex.

Now since each sample of bark was divided into three zones and since the percentage of ray, sieve-tube and cortical tissues in each of the zones can be estimated from the data from each sample, a set of three simultaneous equations can be drawn up, the solution of which will give *estimated concentrations* in sieve-tubes, ray tissues, and cortex. An example will make the calculation clear. The data given refer to one of the four samples.

		As per cent. of Total Area less Fibres.			
Zone.	Fresh wt. as Fraction of Total.	Sieve-Tubes.	Ray Tissues.	Cortex.	Asparagine Concentration.
Inner	0·310	48·6	51·4	—	346·4
Middle	0·305	18·8	80·9	0·3	392·5
Outer	0·385	2·2	32·1	65·7	213·6

The three simultaneous equations are:

$$0{\cdot}486\,S + 0{\cdot}514\,R = 346{\cdot}4$$
$$0{\cdot}188\,S + 0{\cdot}809\,R + 0{\cdot}3\,C = 392{\cdot}5$$
$$0{\cdot}022\,S + 0{\cdot}321\,R + 0{\cdot}657\,C = 213{\cdot}6$$

where S, R, and C represent concentrations in sieve-tubes, rays, and cortex respectively. In this case the solution is

$$S = 265{\cdot}3,\ R = 423{\cdot}0,\ C = 109{\cdot}5.$$

While it is clear that the results for any one sample must be interpreted with great caution, since the data are only just sufficient for the exact solution, leaving no degrees for freedom for any estimate of error, any general conclusion may be tested by comparing the results for the four parallel samples. In applying this type of statistical analysis to the results for sugars in the earlier paper, the two or three parallel samples were first combined, and there were thus six or nine figures available for the calculation of two constants since classification was made into sieve-tubes and bark parenchyma only. The data could thus be treated by the correlation method, which gave at the same time the best value for the constants and an estimate of error. A similar method could have been followed here, using, however, the method of partial correlations, since there are three constants; but it seemed simpler to calculate for each sample separately and then compare the results. The results are given on the left of Table XIII.

TABLE XIII.

Estimated Concentrations of Sugars and Nitrogenous Compounds in the Bark Tissues.

Classification.

	(*a*) Into Three Tissues.						(*b*) Into Two Tissues.			
	Sieve-Tubes.		Rays.		Cortex.		Sieve-Tubes.		Parenchyma.	
	Mean.	Std. Dev.	Mean.	Std. Dev.	Mean.	Std. Dev.	Mean.	Std. Dev.	Mean.	Std. Dev.
Total N . .	988	*26·3*	722	*15·2*	694	*28·8*	1,004	*40·8*	703	*11·22*
Protein N . .	465	*50·7*	320	*32·1*	503	*43·7*	325	*51·7*	409	*13·71*
Total Cryst. N .	523	*26·3*	402	*15·2*	191	*28·8*	679	*18·4*	294	*6·06*
Asparagine N .	297	*24·3*	356	*21·3*	154	*16·7*	428	*21·0*	261	*5·50*
Amino-acid N .	73	*11·1*	11·7	*3·24*	15·4	*3·02*	66·7	*5·64*	12·3	*1·70*
Residual N + Nitrate N .	153	*19·0*	34·3	*4·15*	21·6	*3·94*	184·3	*28·4*	20·7	*8·26*
Sucrose . .	4·28	*0·46*	1·03	*0·32*	0·45	*0·41*	4·67	*0·423*	0·76	*0·112*
Reducing Sugars	2·03	*0·56*	4·01	*0·37*	5·04	*0·27*	1·28	*0·454*	4·43	*0·053*

The variation between the estimates from different samples is fairly large but, testing the data by means of the standard deviations, certain

generalizations seem possible. These will naturally apply only to the particular population of stems handled, and only in so far as the method of analysis is valid. Several types of distribution can be distinguished. The most interesting is that shown by sucrose, amino-acids, and the residual N + nitrate N fraction. These compounds have high concentrations in the sieve-tubes and low concentrations in ray tissues and cortex. The difference between the sieve-tube concentration and the concentration in rays and cortex is fully significant, but there is no significant difference between rays and cortex. Reducing sugars provide almost exactly the reverse case—a low concentration in the sieve-tubes and significantly higher concentrations in the ray tissues and the cortex. The difference between the cortex and the rays, though smaller than that between rays and sieve-tubes, is, however, statistically significant.

A third type of distribution is that given by asparagine. Here the sieve-tubes have nearly double the concentration in the cortex, but the ray tissues have a higher concentration still. The difference between sieve-tubes and cortex is fully significant; the excess of the ray tissues over the sieve-tubes is not so certain; the observed excess might occur by chance in rather more than 5 per cent. of cases. The fourth type of distribution is that of protein N, which has a concentration in the rays significantly lower than that in either sieve-tubes or cortex.

On the right of Table XIII are given the results obtained by classifying into two tissues only, viz. sieve-tubes and parenchyma. The procedure followed is that used in the earlier work on carbohydrate transport. It will be seen that, for compounds which follow the first type of distribution described above, i.e. sucrose, amino-acids, and the residual N + nitrate N fraction, the estimates obtained are very much the same as those obtained from the three tissue classification. For compounds following the second type of distribution, such as reducing sugars and total crystalloid N, there is some distortion in using the simpler classification, but the distortion is relatively slight. In dealing with sugars therefore, the simpler form of analysis which was used in the earlier paper would seem to be justified, and the same may be true for amino-acids and the residual N + nitrate N fraction. Only in the case of asparagine and protein N does the simpler form of analysis seriously distort the results.

The results may be summed up as follows. For the following compounds, namely sucrose, reducing sugars, amino-acids, and the residual N + nitrate N [1] fraction, the radial distribution of concentrations in the bark can be almost entirely explained by the radial distribution of the sieve-tubes. For these compounds the most important inter-tissue difference would seem

[1] The characteristic form of the radial distribution of the residual N + nitrate N fraction is probably due to the residual N fraction only for, as we noted earlier (p. 637), the radial gradient in nitrate is very small.

to be that between the concentration in the sieve-tubes and the concentration in other tissues. For protein N and asparagine, on the other hand, the distribution of the ray tissues also must be taken into account. The very interesting question arises whether this classification of the compounds on the basis of radial distribution may not correspond to a classification into compounds mainly concerned in longitudinal transport and compounds mainly concerned in local storage. The fact that amino-acids and residual N show a distribution like that of sucrose argues in favour of a transport function, while the distribution of asparagine suggests a storage function.

An alternative way of presenting the results lies in considering the composition of the total nitrogen in the three tissues. This is shown in Table XIV.

TABLE XIV.

Percentage Composition of the Total Nitrogen in Sieve-tubes, Rays, and Cortex.

	Per cent. of Total N.		Per cent. of Total Crystalloid N.		
	Protein N.	Crystalloid N.	Asparagine N.	Amino-acid N.	Residual N + Nitrate N.
Sieve-tubes	47·0	53·0	56·7	14·1	29·2
Ray Tissues	44·4	55·6	88·4	2·9	8·7
Cortex	72·5	27·5	80·4	8·1	11·5

It will be seen that whereas asparagine N forms nearly 90 per cent. of the total crystalloid N in the rays, in the sieve-tubes it forms only a little more than half the total crystalloid N.

(3) *Experiment 5.*

One further experiment on radial gradients was carried out. This was concerned with following the effects of ringing, and the results will be presented in the section dealing with ringing. In view, however, of the importance of the general phenomena of radial distribution of concentrations in the bark, the mean values of the estimated concentrations for the three tissues in that experiment are given below (Table XV) for comparison with the results just described.

The mean concentrations given in the table are averages of four samples from the same region of stem. Two of the samples were from plants having a ring above the sampled region of stem, while the other two were from plants having a ring below the sampled region of stem.

For sugars and for asparagine, the results are very similar to those in the experiment just recorded and confirm the generalizations made. The chief difference is in the protein N, which exists here in greatest concentration in the rays.

The relative accumulation of the asparagine in the rays is even more marked in this experiment than in the previous one. It will be noted that asparagine N now forms less than half the crystalloid N in the sieve-tubes. Amino N was not estimated, but the distribution of amino-acid + residual N + nitrate N is similar in the two experiments. The concentration in the sieve-tubes is definitely very much higher than in the other tissues, and there is very little difference between rays and cortex. Apart from the alteration in the distribution of protein N, the significance of which is not clear, the results confirm the general conclusions already reached.

TABLE XV.

Estimated Concentrations in the Bark Tissues.

(A) *Mean Concentrations.*

	Sieve-tubes.	Rays.	Cortex.
Total N . .	706·6	768·9	539·7
Protein N . .	376·6	478·7	368·1
Total Cryst. N .	330·0	290·2	171·6
Asparagine N .	151·1	218·7	113·4
Amino-acid + Residual N + Nitrate N .	178·9	71·5	58·2
Sucrose . .	3·418	0·927	0·266
Reducing Sugars .	0·808	2·130	2·910

(B) *Percentage Composition.*

	Per cent. of Total N		Per cent. of Crystalloid N	
	Protein N.	Crystalloid N.	Asparagine N.	Amino-acid + Residual N + Nitrate N.
Sieve-tubes	53·3	46·7	45·8	54·2
Ray tissues	62·3	37·7	75·4	24·6
Cortex	68·2	31·8	66·1	33·9

SECTION 5. CONCENTRATIONS IN LEAF-PARENCHYMA, LEAF-MIDRIB, AND PETIOLE.

(EXPERIMENT 13).

In order to complete the general picture of the distribution of nitrogen concentrations in the vegetative plant, observations were made on concentrations in leaf-parenchyma, midrib and petiole.

(1) *Procedure.* The leaves chosen were fully mature leaves on the main-axis near the top of the plant. Two samples each of 200 leaves were taken. The five main veins of each leaf were carefully cut out and separated from the green tissue. This sample of the veins will be termed *midrib* —the remaining tissue, which of course included the smaller veins, will be

termed *parenchyma*. On account of the large amount of sap needed for nitrogen determinations it was necessary to bulk the midrib material from the two samples. The two samples of petioles and parenchyma were, however, analysed separately.

The material was collected at 1.0 p.m. on February 23, 1928. The determinations made were: total N, crystalloid N, asparagine N, amino N, nitrate N, ammonia N, sucrose, reducing sugars, and hydrogen-ion concentration.

(2) *Results.* Table XVI shows the concentrations found. In the case of petioles and parenchyma the figures given are the means for the two samples.

TABLE XVI.

(a) *Concentrations in Leaf-parenchyma, Leaf-midrib, and Petiole.*

	Parenchyma.	Midrib.	Petiole.
Total N	1594·1	532·4	351·6
Protein N	1436·7	414·5	199·3
Total Cryst. N	157·4	117·9	152·3
Organic Cryst. N	150·5	78·25	64·2
Asparagine N	9·63	7·69	15·0
Amino-acid N	21·95	9·33	7·23
Residual N	118·85	61·23	41·96
Nitrate N	3·54	38·66	86·47
Ammonia N	3·39	0·99	1·66
Total Sugars	1·617	2·509	1·996
Sucrose	0·345	0·877	0·879
Reducing Sugars	1·272	1·632	1·118
Hydrogen-ion (pH)	6·215	5·310	5·370

(b) *Relative Concentrations.*

	Parenchyma.	Midrib.	Petiole.
Sucrose as per cent. of Total Sugars	21·34	34·95	44·04
Protein N as per cent. of Total N	90·13	79·35	56·71
Organic Cryst. N as per cent. of Total Cryst. N	95·62	66·4	42·2
Nitrate N as per cent. of Total Cryst. N	2·25	32·79	56·78
Asparagine N as per cent. of Organic Cryst. N	6·40	9·82	23·4
Amino-acid N " " " "	14·60	11·92	11·28
Residual N " " " "	79·0	78·26	65·32

It will be seen that whereas protein N increases greatly as we pass from petiole to midrib, and from midrib to parenchyma, total crystalloid N shows very much the same concentration in all three tissues. The organic crystalloid N, however, exhibits a sharp fall from parenchyma to midrib and a further fall from midrib to petiole. This gradient out of the leaf is almost entirely due to residual N. Amino-acid N, with a concentration level about one-sixth of that for residual N, shows a very similar gradient. The concentration of asparagine N, however, is not appreciably different in parenchyma and midrib but is greater in the petiole.

Especially noteworthy is the steep gradient of nitrate N from the petiole through the midrib to the parenchyma. It is interesting to note that the concentration of nitrate N in the vessel sap[1] of the wood is of about the same order as that found here in the lamina, viz., 3·54 mg. per 100 grm. water.

The gradients of nitrate N from petiole to lamina, and of organic crystalloid N from lamina to petiole are, of course, what might be expected if nitrates entered the lamina and organic N left it by a process analogous to diffusion. As nitrates, however, almost certainly enter the leaf with the transpiration current and not by any process analogous to diffusion, it seems probable that the nitrate N gradient is merely an expression of the fact that the synthesis of organic nitrogen is more rapid in the lamina than the midrib, and more rapid in the latter than the petiole. In consequence of this gradient of nitrate N from the petiole to the lamina, there may of course be a movement of nitrate N into the lamina by a process analogous to diffusion as well as by a mass movement in the transpiration current.

The outwardly directed gradient in organic crystalloid N, on the other hand, may well be an indication of a gradient in the same direction in the actual channels of transport from lamina to petiole. If this is the case then residual N, and possibly amino-acids also, may furnish the 'head' for the export of organic nitrogen from the leaf. Militating against this view is the fact that the concentration of residual N in the sieve-tubes of the bark may apparently be very high (Tables XIII and XV). No estimates of the concentration in the sieve-tubes are available for the present experiment, consequently we cannot exclude the possibility of a gradient in residual N from the assimilating cell to the sieve-tubes of the bark.

If this picture is true, the export of organic nitrogen from the leaf forms an interesting contrast to the export of sugars. From our earlier work on carbohydrate transport in the cotton plant we concluded that the 'head' in the leaf is composed of reducing sugars, and that these move along a concentration gradient from parenchyma to sieve-tube. In the sieve-tubes the reducing sugars are converted to sucrose, and from this point the 'head' for transport of sugar is mainly sucrose. In the case of organic nitrogen, on the other hand, it seems possible that residual N may be the 'head' for transport throughout the plant. The increase in the proportion of asparagine as one passes from parenchyma to midrib and petiole (Table XVI B) would thus be a phenomenon of storage and not, as is the case with the parallel increase in proportion of sucrose, the establishment of a transport head. That these different interpretations of the behaviour of sucrose and of asparagine respectively are not improbable is suggested by the fact that, whereas, in the bark, the proportion of sucrose generally diminishes (10) as we pass

[1] Vessel sap obtained by displacement method (cf. 9).

down the stem away from the foliage region, the proportion of asparagine shows a marked increase (Table IX). The proportion of sucrose increases from leaf to bark but diminishes from that point onwards: the proportion of asparagine increases from leaf to bark but increases still further as we pass down the stem.

Section 6. Discussion.

It will be evident that the data on longitudinal gradients of nitrogenous substances are still somewhat fragmentary. Certain facts of a rather unexpected nature have, however, been revealed. Thus it seems clear that in contrast to the behaviour of carbohydrates the downward movement of nitrogen through the bark from the leaves to the roots occurs in the presence of a well-defined negative gradient of organic crystalloid N. As the negative gradients in asparagine and amino-acids seem more than adequate to account for the organic crystalloid N gradient, a small positive gradient in residual N or some part of it may perhaps be inferred. Now if nitrogen is transported, like carbohydrates, by a process analogous to diffusion, it might be anticipated that though the gradients in the bark as a whole are either negative or slightly positive, yet in the actual channels of transport there would be a positive gradient in, at all events, the mobile compound or compounds. The presence of positive longitudinal gradients in organic N in wood and leaf lends some support to the view.

If there is a positive gradient in the mobile compound or compounds in the sieve-tubes which is masked by negative gradients of storage nitrogen in the other tissues of the bark, subdivision of the bark into two zones, and examination of the gradients in the inner and outer halves, ought to reveal a relatively less marked negative gradient in the inner half. We have found, however, that the gradients in the two halves are approximately the same, and consequently that there is no suggestion of a positive gradient in the sieve-tubes which is masked by negative gradients of storage nitrogen elsewhere. For substances, like amino-acids and the residual N fraction, whose concentration is about the same in the cortex and the rays, subdivision of the bark into two halves ought to show up any masking of a positive gradient in the sieve-tubes by a negative gradient in the rays and cortex. For asparagine, on the other hand, which has a greater concentration in the rays than the cortex, subdivision of the bark into two halves need not reveal the masking of a positive gradient in the sieve-tubes.

To sum up, while our observations on longitudinal gradients in the bark renders it very improbable that there are well-defined positive gradients in either amino-acids or residual N in the sieve-tubes, and not very probable that there is a positive gradient in asparagine, it must be remembered that a positive gradient in the sieve-tube might be masked by a negative one in the companion cells, and moreover that the gradient in the sieve-tube

itself may consist of two components, a dynamic gradient in translocatory nitrogen superimposed on a steeper reverse gradient of relatively immobile or storage nitrogen.

Section 7. Summary.

1. The resemblance in gross phenomena between nitrogen transport and carbohydrate transport suggests that, for nitrogen as well as for carbohydrate transport, there should be a gradient basis. Yet while the downward movement both of nitrogen and of carbohydrates, from the foliage region towards the roots, occurs in the bark, the vertical concentration gradients in the bark of total sugars and of organic crystalloid N are in opposite directions. The movement of carbohydrate is down a gradient of total sugars, while the movement of nitrogen is against a gradient of organic crystalloid nitrogen.

2. The leaves, which supply organic nitrogen to the bark, and the wood which is in dynamic equilibrium with the bark, show, however, vertical gradients of organic crystalloid nitrogen in the direction of downward movement, i. e. the concentration is higher in the apical region of the plant than in the basal region. The leaves show also a positive vertical gradient in total sugars, but the wood shows no consistent vertical sugar gradient. These observations support the conception of a positive gradient of mobile nitrogen in the bark, for the mobile nitrogen in leaves and in wood should be in dynamic equilibrium with such a gradient.

3. If there is a gradient basis for longitudinal transport of nitrogen, the positive gradient of mobile nitrogen in the bark must be in some way masked, for the net gradient of organic crystalloid nitrogen is negative. There may be *chemical masking*, the positive gradient of the mobile chemical compound being outweighed by negative gradients of compounds which are immobile; or there may be *regional masking*, the positive gradient of the mobile compound or compounds in the actual channels of transport being obscured by negative gradients of the same compounds in the other tissues of the bark.

4. As to the first possibility (chemical masking), analysis of the crystalloid N fractions established definite negative gradients, in the bark as a whole, of asparagine and amino-acids. These gradients are more than adequate to account for the net negative gradient in organic crystalloid N, and a small positive gradient in residual N is inferred. This gradient could not, however, be definitely established.

5. As to the second possibility (regional masking) it was found that the negative gradients in organic crystalloid N and in the asparagine and amino-acid fractions are even greater in the inner half of the bark, which contains most of the sieve-tubes, than in the outer half, which is composed mainly of ray tissues and cortex. Moreover, the small positive gradient in residual N

is no more definite in the inner than in the outer half. Unless the negative gradients in the ray tissues are much steeper than those in the cortex, this evidence is strongly against the theory of positive gradients in the sieve-tube tissues, masked by negative gradients in the storage tissues. Masking by negative gradients in the companion cells cannot, however, be excluded, and there is the further possibility that only part of the crystalloid nitrogen in the sieve-tube is mobile, part being held by adsorption or otherwise rendered immobile.

6. The radial distribution of the crystalloid nitrogen fractions across the bark indicates that amino-acids and residual nitrogen, like sucrose, are present mainly in the sieve-tubes. Asparagine, however, though present in high concentration in the sieve-tube, has its highest concentration in the rays. This distribution suggests that amino-acids or residual N may be concerned mainly with transport, while asparagine is concerned mainly with storage.

7. The concentration of organic crystalloid N falls as we pass from the leaf-parenchyma to the main veins and from the main veins to the petiole. The gradient of nitrate N is in the opposite direction. The gradient of organic crystalloid nitrogen out of the leaf is due mainly to residual nitrogen, but there is also a small gradient of amino-acids in the same direction. The asparagine gradient, however, is in the reverse direction. The proportion of residual nitrogen diminishes as we pass from leaf-parenchyma through the main veins and petiole to the bark, and diminishes still further as we pass down the bark of the main-axis. Parallel with this decrease in the proportion of residual N there is an increase in the proportion of asparagine.

The authors wish to record their indebtedness to Professor F. Hardy and to Mr. R. Follet Smith for their advice and assistance.

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A Stemphylium Saltant of an Alternaria.

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With Plate XIV and four Figures in the Text.

THE genus *Alternaria*, as now generally accepted, is characterized by dark-coloured conidia which are muriform, though sometimes with only a few longitudinal septa, usually obclavate, but may also be ovate, fusiform or cylindrical, always with a more or less pointed apex which may be drawn out into a beak; under favourable conditions they are borne in chains. The conidiophores are coloured, mostly simple, but may be branched, erect or subdecumbent, straight or jointed, with a scar at each geniculation.

Stemphylium, on the other hand, has typically oval or oblong, muriform, dark-coloured conidia, though spherical ones with transverse and longitudinal walls dividing them are also seen; they may sometimes be borne in short chains, and occasionally they develop a beak, thus closely resembling those of *Alternaria*. The conidiophores are either short, hyaline, and unbranched, or their growth may be continued at the apex, when the terminal spore becomes lateral, or a branch may be given off a short distance below the apex, when the terminal conidium becomes lateral on a short branch. Both the latter processes may be repeated a number of times.

In comparing these genera, it is noticeable that as regards spore shape, cultures of *Alternaria* readily yield spores like those of *Stemphylium*, whilst in *Stemphylium* conidia not infrequently occur which exactly correspond with those of *Alternaria*. Bolle (1), in her recent paper on the Phaeodictyae, remarks (on p. 52) that on one occasion she encountered a *Stemphylium* which might easily have been confused with an *Alternaria*, as there were few or no round conidia present, and always some of *A. cheiranthi* admixed. There is no hard and fast line between the two genera, but the predominance of one type of spore is usually so clearly marked that there is no difficulty in deciding to which genus a fungus belongs from the spore shape alone. Similarly with regard to the catenulation of the spores,

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certain species of *Alternaria* with long beaks, e. g., *A. macrospora*, have only two or three conidia in a chain at the most, whilst in some species of *Stemphylium* chains of two or three conidia are found, but no species with chains of twenty or more conidia, as in *A. tenuis*, has ever been recorded for *Stemphylium*. In each genus a circular scar is left where the conidium leaves the conidiophore, though in *Alternaria*, as the conidia are borne in chains, there are naturally far fewer scars than in *Stemphylium*.

From this brief comparison, therefore, it is evident that while the two genera are easily recognizable, a close connexion exists between them, and the saltation described below shows that the relationship is even closer than has been anticipated.

The saltating fungus, which has been the subject of study, occurred on a Petri dish, on which isolations were being made from grapes received by the Imperial Bureau of Mycology from Australia. Miss Hellinger, of the Zionist Agricultural Experiment Station, Palestine, who was making the isolations, obtained a growth of *Stemphylium* in one of her plates, and Mr. Mason, Assistant Mycologist of the Imperial Bureau, drew my attention to the fact that one portion of the colony appeared to be producing a growth of the *Alternaria* type. The plate was contaminated with bacteria and other fungi, and in the region where the *Alternaria* occurred, the *Stemphylium* mycelium was swollen up, dark coloured, and evidently adversely affected either by the thin, partially desiccated agar, or by the growth of an adjacent bacterial colony, or by both.

Single conidia of the *Alternaria* were transferred on the dry point of a needle to Richards's agar in Petri dishes, and after some days' incubation at 22° C. some of the *Alternaria* colonies developed a growth of *Stemphylium*. From one of these saltating colonies tube cultures of the *Alternaria* and the *Stemphylium* were made, but the former, after some days, saltated to the latter. Also, single conidia of the *Alternaria* were taken from the saltating colonies and placed on Richards's agar in Petri dishes—one conidium being placed just on the surface of the agar in a marked circle in the centre of each dish, the sides of which were then plugged with cotton wool to prevent contamination. Richards's agar contains a flocculent precipitate, and in pouring only the clear supernatant agar was decanted to give as clear a medium as possible. Some of the conidia did not germinate; a few gave only *Alternaria* (the agar in these dishes was shallow and the cultures dried up prematurely); a few others gave only *Stemphylium*; while most gave at first *Alternaria*, which after some days showed *Stemphylium* growth in one or more regions. Sub-cultures of single *Alternaria* conidia were again made from the saltating colonies and the process repeated, so that altogether upwards of twenty single spore cultures of the *Alternaria* have produced the *Stemphylium* saltant. Richards's agar was used in nearly all cases, though saltation occurred also on potato dextrose

agar (a medium recommended by Brown for avoiding saltations of *Fusarium*), Dox agar, bean agar, malt extract agar, and on oat agar.

The saltation usually became apparent on Richards's agar when the colony was about 2 cm. in diameter, generally after about five or six days incubation at 22° C., and appeared as one or two rapidly growing mycelial branches at the edge of the colony. These soon produced conidia of the *Stemphylium* type, and their subsequent growth often gave rise to a bulge on one side of the colony (Pl. XIV, Fig. 1), which ultimately outgrew the *Alternaria* altogether. In some dishes the *Stemphylium* completely surrounded the *Alternaria* colony, which did not reach more than 2 by 4 cm. altogether. Sometimes only one part of the *Alternaria* colony saltated, sometimes three or four, usually in irregular order, one after the other. Once, on bean agar, eight saltations were observed. Not infrequently, sectors gradually appeared as small, broad-based triangular areas, with the apex at some distance from the centre of the colony. The *Stemphylium* grew just about twice as quickly as the *Alternaria*, e.g., a single spore colony of the former on Richards's agar reached 4·5 cm. in diameter in six days, while four of the latter only reached 2·2, 2·3, 2·4, and 2·5 cm. respectively, in the same time. Only one type of saltant was produced, and this showed no signs whatever of reverting to the *Alternaria*. It may be remarked, however, that even if the *Stemphylium* should saltate back to the *Alternaria*, the latter growth would be immediately obliterated by the more rapid growth of the *Stemphylium*. An attempt was made to induce the *Stemphylium* to saltate back to the *Alternaria* by making single spore cultures of the *Stemphylium* on Richards's agar. It was thought possible that the spore of the saltant might give rise directly to a pure *Alternaria* colony in the same way as occasional *Alternaria* spores produce *Stemphylium* apparently without any development of *Alternaria*; the *Stemphylium* would thus have been eliminated altogether. None of sixteen cultures in Petri dishes, however, showed any sign of an *Alternaria* reversion. Further, in an attempt to reproduce the condition in Miss Hellinger's Petri dish, eight of the cultures were made on sloped agar in the hope that the gradual desiccation of the agar might result in the slowing down of the *Stemphylium* growth and permit the *Alternaria*, if present, to manifest itself; whilst in four of the Petri dishes, in addition to the single spore of *Stemphylium*, an inoculation was made about 2 cm. distant with the bacterial contamination from Miss Hellinger's dish. Both these methods, however, were unsuccessful in their aim.

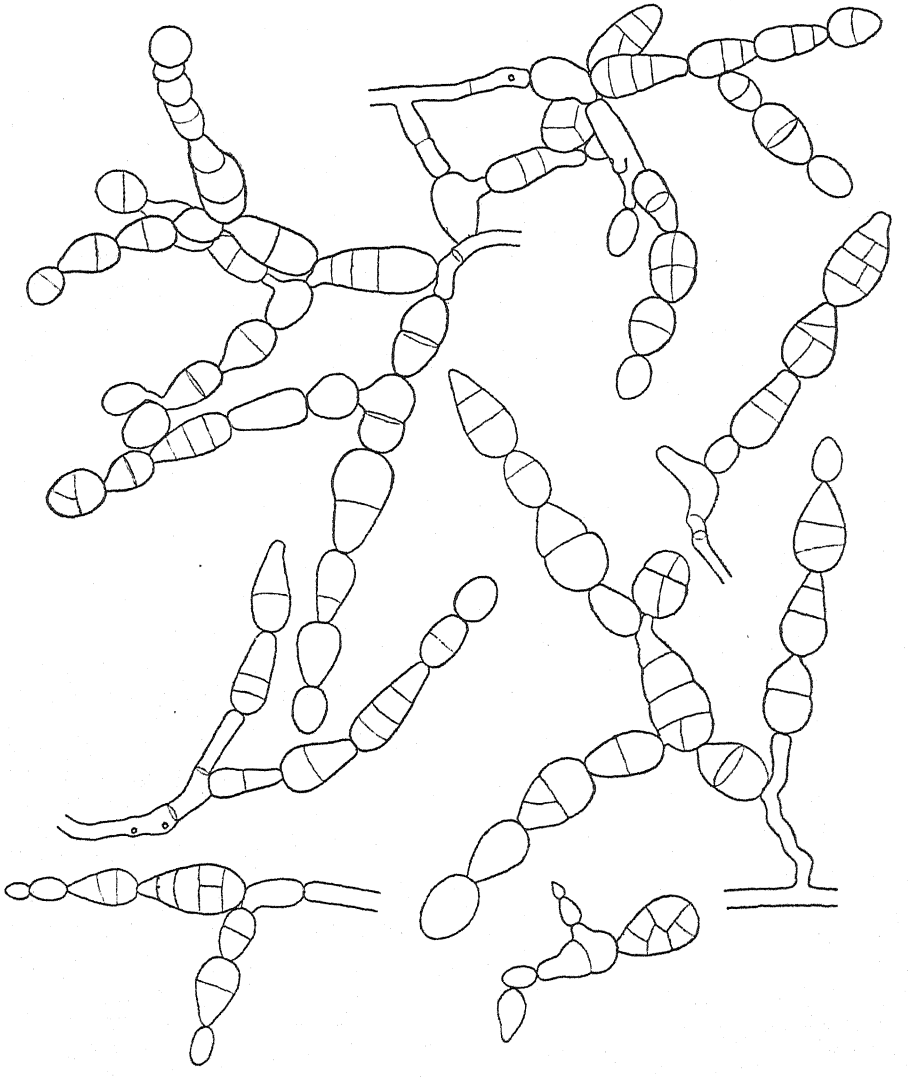
This type of saltation is similar to that described by Mohendra (6) for a strain of *A. tenuis*, which almost invariably showed a sectored appearance when the cultures reached a certain size, whilst the saltant did not show a ' tendency to throw out sectors so often '. Crabill (3) recorded a somewhat comparable type of saltation in a fungus referred to as *Coniothyrium pirinum*

which at intervals produced a stable, poorly fructifying saltant from the abundantly fructifying parent strain; and Stevens (8) also encountered saltants of *Helminthosporium* which maintained their characters through a succession of transfers, and through dilution platings of the conidia. Others have also recorded non-reversible saltations, though the author knows of no one who has mentioned a saltating species which almost invariably throws a form belonging to another genus.

On Richards's agar, in Petri dishes, the *Alternaria* produces at first a somewhat spreading, roughly circular colony, which later becomes a compact, felt-like growth in the centre, with a thinner margin. In colour, a colony of about 5 cm. in diameter is brownish-olive (Ridgway [1]) in the centre, and dark greenish-olive at the edge, whilst the reverse side is olivaceous-black; in older colonies it is slightly darker. On other media the type of growth is somewhat different, e.g., on bean agar and malt extract agar, it is more compact and regular, whilst on Dox it is radiating and the long strands of mycelium remain distinct and gradually increase in thickness to form rope-like branches. The colour, too, varies slightly according to the medium; on bean agar it is deep to dark greyish-olive; on malt extract agar it is dull greenish-black. Not infrequently a small whitish aerial growth is visible at the original place of inoculation, or in one or more isolated spots on the surface of the colony, probably white variants as described by Caldis and Coons (2) for various fungi, or even saltants as recorded by Mohendra (6). The conidia are borne in long chains which show considerable branching; the branches frequently develop as outgrowths at the apex of a conidium immediately below its junction with the one above (Pl. XIV, Fig. 2, and Text-fig. 1). The conidia vary much in appearance (Pl. XIV, Fig. 2); they may be closely similar to those of *A. tenuis* in shape, dark olive-green in colour, closely warted with usually 0 to 3 cross septa, though as many as six have been counted, with 0 to 3 longitudinal walls, and with a definite beak; or they may be spherical or oblong, and like those of the *Stemphylium* saltant only smaller; some are small, unicellular, spherical or elongated, hyaline or slightly tinted, and sometimes with irregular wart-like incrustations over certain areas; and others are spherical, dark-coloured, warted, unicellular or with one or more dividing walls. All intermediate stages between these forms can be recognized. There is a distinct tendency for the conidia in any one chain to resemble each other, a tendency which has also been observed in *A. circinans*. When the fungus is growing vigorously the conidia are usually less variable, better developed both in shape and structure, and at their best present the appearance of a true *Alternaria*, but sometimes the chains of spores are most irregular, small hyaline spores

[1] Ridgway's nomenclature for colours is used throughout this paper.

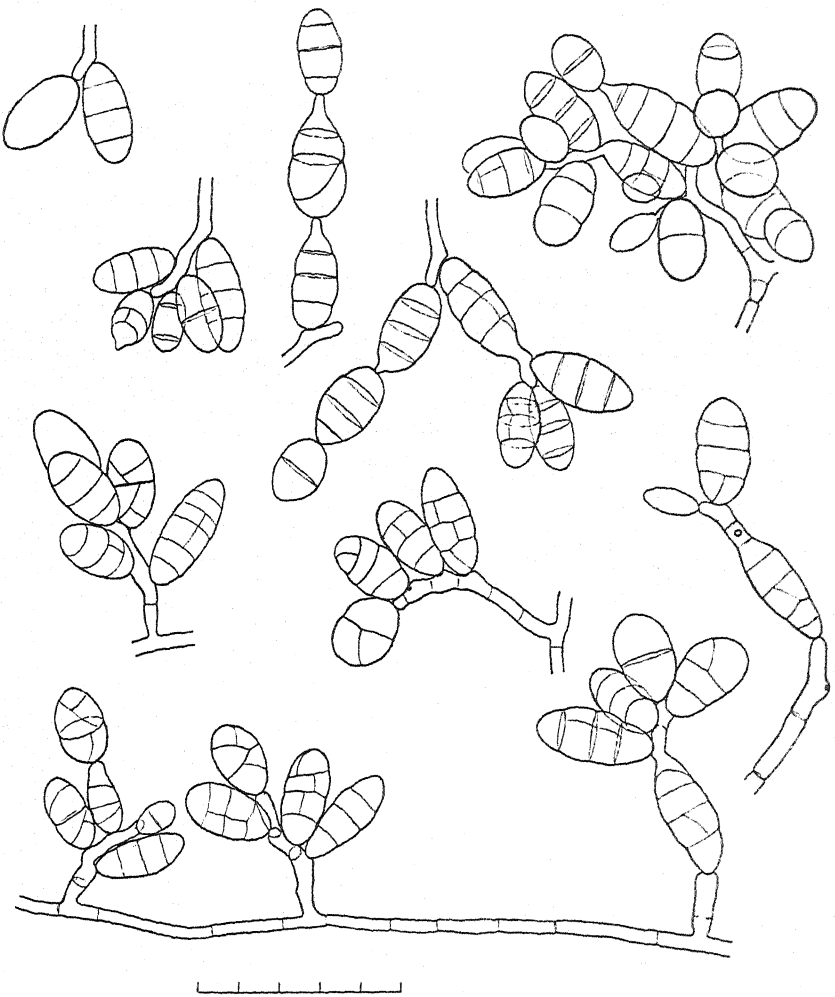
being interspersed with dark-coloured, larger ones. The variability of the conidia appears to be somewhat reduced by cultivation on potato dextrose



TEXT-FIG. 1. Conidia and conidiophores of a species of *Alternaria* isolated from grapes. × 550.

agar, and is much less in some cultures than in others, associated perhaps with the vigour of growth. The conidia (including the beak) measured 13 to 26 by 6 to 14 μ in one single spore culture, 13 to 28 by 7 to 13 μ in another, and 10 to 30 by 8 to 15 μ in a third, on Richards's agar, twenty conidia of each being measured; on bean agar, the measurements of a similar number were 8 to 30 (once 37) by 8 to 16 μ. The mycelium when

submerged in agar often produces chains of small hyaline spores, usually unicellular but occasionally divided by cross walls, oblong, but more often

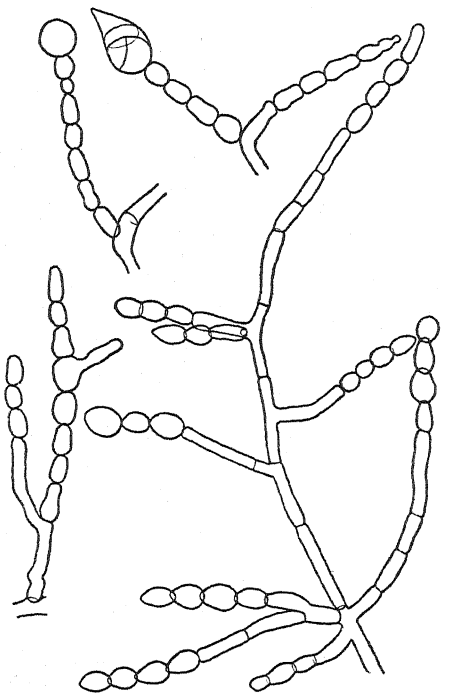


TEXT-FIG. 2. Conidia and conidiophores of a *Stemphylium* saltant of the *Alternaria* shown in Fig. 1. ×550.

slightly pointed towards the tip (Text-fig. 3). Sometimes the terminal cells are enlarged, but usually the chains simply lengthen but do not develop further. These chains are borne either terminally on branches of the mycelium or laterally on such branches. They are particularly interesting in comparison with corresponding structures in the saltant.

The *Stemphylium* saltant can be clearly distinguished from the *Alternaria* by the darker colour of its colony, which, on Richards's agar, is olivaceous black (2) at the centre, shading to deep olive at the edge. This

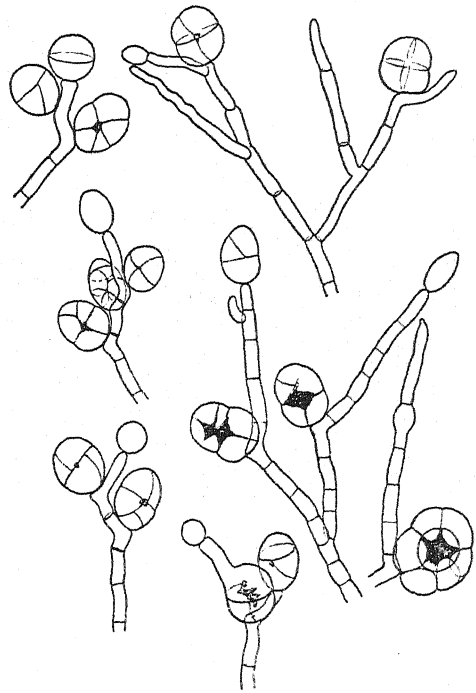
difference in colour marks out the extent of the *Stemphylium* growth in saltating colonies of *Alternaria*. Its spores (Pl. XIV, Fig. 3, and Text-fig. 2) are of the normal *Stemphylium* shape, oval, muriform, sometimes with a small point, and coarsely warted; they have from 0 to 5 (mostly 3)



TEXT-FIG. 3. Submerged conidia and conidiophores of the *Alternaria*. ×440.

cross septa with a number (usually 2 in 3-cross-septate spores) of longitudinal ones, but not infrequently they are obliquely divided, sometimes only in part (an oblique septum usually having another meeting it approximately at right angles), or a median transverse septum may be followed by vertical ones. Conidia of the *Alternaria* type occasionally occur. In colour the conidia vary from light to dark olive, and sometimes are so opaque that the cross walls cannot be discerned. They measured 15 to 38 by 11 to 17 μ for one strain of the saltant, and 11 to 37 by 9 to 21 μ for another. Chains of two or three conidia are not uncommon, but the maximum number observed in a chain did not exceed four. The conidia are borne in botryose heads (Text-fig. 2), and as each of the conidia directly attached to the conidiophore may bear one or more conidia itself, the clusters often contain a considerable number of spores. Sometimes the distal end of the spore is prolonged to form itself a short conidiophore, which again bears a small head of spores. The hyphae, when immersed in

the agar, bear hyaline spores (which in old cultures become very slightly olive), usually roughly spherical in outline with vertical and transverse cross walls, at the intersection of which a small bubble of gas appears as a dark object in the centre of the conidium (Text-fig. 4). The surface



TEXT-FIG. 4. Submerged conidia and conidiophores of the *Stemphylium* saltant. ×440.

of these spores is smooth. They arise terminally on a hypha which afterwards continues its growth round the side of the conidium to form a second terminal conidium. This process may again be repeated, so that finally a conidiophore with a number of lateral spores and one younger terminal one is produced.

The *Alternaria* is tentatively regarded as a small spored strain of the *A. tenuis* group. The latter term has been used at the Imperial Bureau of Mycology (5) to designate forms having obclavate conidia, borne in long chains, the majority of whose spores have 3 to 5 cross septa, and come within the measurements 20 to 50 by 10 to 14 μ; these measurements need to be extended to 10 to 50 by 10 to 16 μ to include the form studied, while the number of septa in the latter is also fewer.

The *Stemphylium* has been compared with Oudemans's culture of *S. botryosum*, but though the spore measurements agree closely (*S. botryosum* = 9 to 39 by 9 to 22 μ), the conidia of the latter are not borne in such close clusters. A species recently described by Newton (7) as *S. congestum*

(17 to 30 by 12 to 19 μ) appears to be very like the present saltant, though no mention is made in the description of submerged, hyaline conidia. The record of *S. graminis* (15 to 36 by 15 to 18 μ) on rotting apples by Kidd and Beaumont (4), was thought perhaps to refer to the same species, but on examination of a culture of the fungus, kindly forwarded by Mrs. Kidd, it was found that, while closely similar, it differs in the apparent absence of submerged conidia, its slower rate of growth (on Dox 4·8 cm. against 9·3 cm.), its rather more greyish colour (on Dox and malt extract agars) without any tinge of yellow (particularly noticeable at the edges), and its slighter tendency to form chains.

In recent years the opinion of mycologists has tended to regard all saltants of a given species as strains of that species, though Stevens (8) has shown that certain saltants of *Helminthosporium* differ so markedly from the parent as to exceed considerably the usually accepted specific limits. When the saltants come within the generic limits of the parent species, such a course is possible, for the conception of a species can always be widened to include all the saltant forms, but when the saltant falls outside the generic limits of the parent, this procedure is scarcely permissible, for we cannot have one form species divided into two genera ; though, of course, one does not hesitate to regard the separate stages in the life-history of many fungi as species of different genera. The question arises, therefore, whether *Stemphylium* can be maintained as a separate genus if a species of *Alternaria* can give rise to it by saltation. In a purely artificial system of classification, as that of the Fungi Imperfecti is acknowledged to be, a genus that can be easily identified by morphological characters has every right to be maintained, and as we have seen, the genus *Stemphylium* is easily recognized and well established, nor is there the slightest reason to suppose that all its species are *Alternaria* saltants. It cannot, therefore, lightly be discarded. There appear to be three courses open to us, (*a*) still to regard all saltations as strains of the parent species, in which case the *Stemphylium* saltant would become merely a strain of the *Alternaria*, (*b*) to regard marked saltations as good terminological species, and to give them specific names, when the *Stemphylium* saltant would become a distinct species from the *Alternaria*, and would be retained in the genus *Stemphylium*, or (*c*) to regard the saltation described as merely the development of another stage in the life-history of the fungus, and not as a true saltation. If the last-named view is favoured we should expect the *Stemphylium* to be able to give rise to the *Alternaria* stage under favourable conditions. In the original culture the *Alternaria* appeared to come from the *Stemphylium*, and the failure to obtain the former from the latter in the limited number of experiments made, does not eliminate the possibility of the stable *Stemphylium* form giving rise on rare occasions to the unstable *Alternaria* form. Proof is needed, however, that this actually occurs, especially since

non-reversible saltations occur in other fungi. Furthermore, there is a marked difference in the rates of growth of the *Alternaria* and the *Stemphylium*, which rather suggests that the phenomenon described has the nature of a true saltation. The whole matter, however, is obscure, and further data are required to enable a considered opinion to be expressed regarding it.

SUMMARY.

1. A brief comparison is made between the genera *Alternaria* and *Stemphylium*.

2. A species of *Alternaria*, isolated from rotting grapes received from Palestine, repeatedly produced in single spore cultures a saltant form which has been assigned to the genus *Stemphylium*. The saltant has shown so far no reversion to the *Alternaria*. The characters of both the *Alternaria* and its saltant are described.

3. The taxonomic significance of this type of saltation is briefly discussed.

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EXPLANATION OF PLATE XIV.

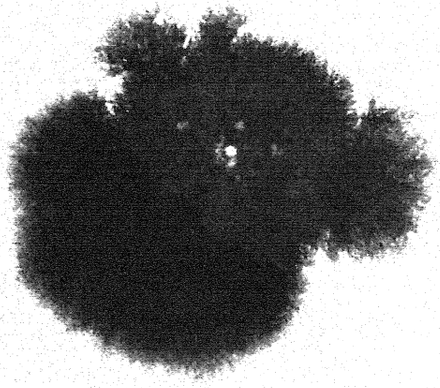
Illustrating Mr. S. P. Wiltshire's paper on a *Stemphylium* Saltant of an *Alternaria*.

All figures and text-figures with the exception of Pl. XIV, Fig. 2, are from cultures on Richards's agar.

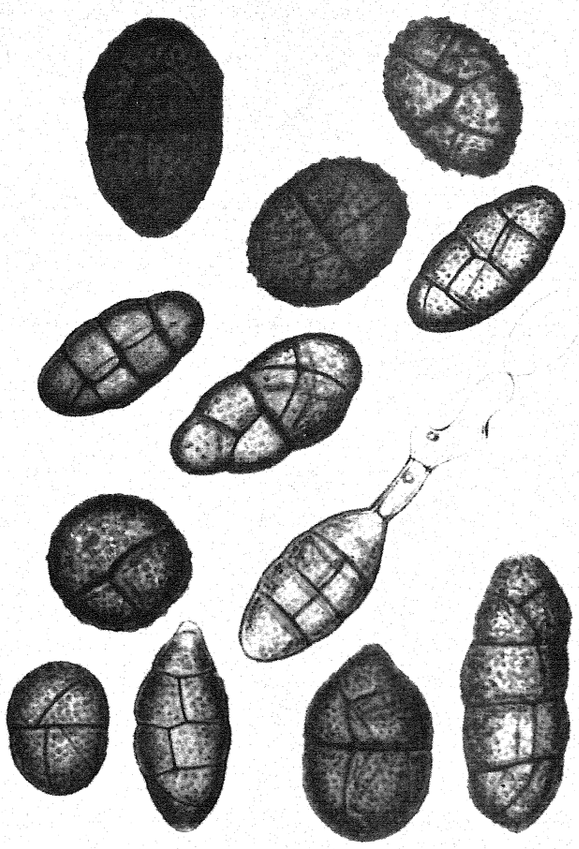
Fig. 1. Single spore culture of an *Alternaria* isolated from grapes showing the central *Alternaria* growth saltating to *Stemphylium* in four places. (Photograph by Mr. G. Atkinson).

Fig. 2. Conidia of the *Alternaria* showing their variation. *x* = conidia from bean agar; *y* = from malt extract agar. × 1120.

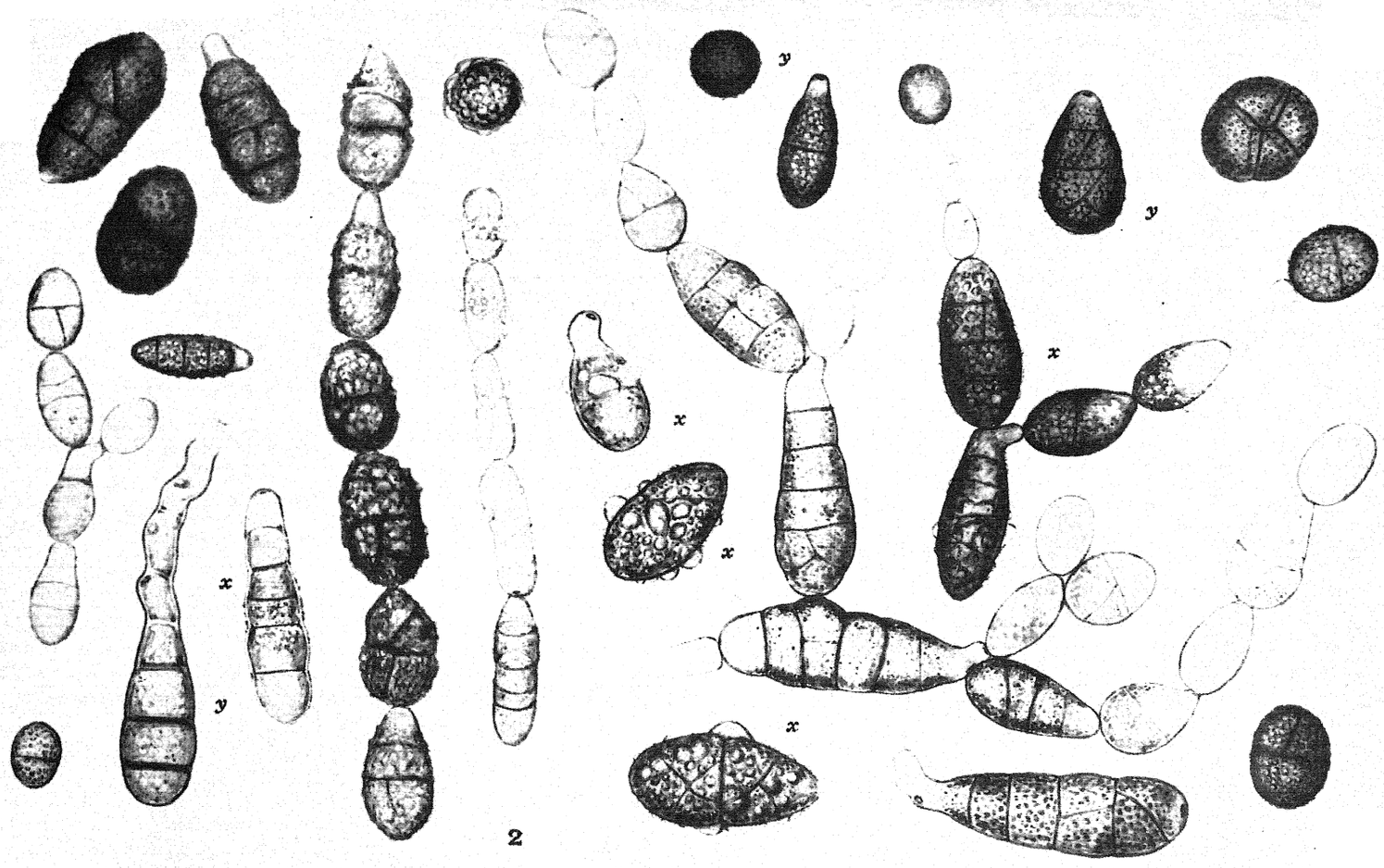
Fig. 3. Conidia of the *Stemphylium* saltant. × 1120.



1



3



2

WILTSHIRE — SALTATION IN ALTERNARIA.

Huth. London.



On Fossil Wood (Dadoxylon Hendriksi, n. sp.) and other Plant-remains from the Clay-Slates of South Cornwall.

BY

WILLIAM H. LANG, F.R.S.

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With Plates XV and XVI.

THE chief specimen to be described in this paper is a piece of partially silicified wood with the complex structure of a typical *Dadoxylon*. Its special botanical interest depends on the geological age of the beds in which it was found. In the Memoirs of the Geological Survey (3, 8) these are regarded as Ordovician, and the youngest period to which they have so far been referred by geologists appears to be that of the Lower Devonian (6, 7). The one well-preserved specimen was found after prolonged and rather fruitless study of numerous imperfectly preserved fragments of plants that are abundant in these rocks. Two or three specimens have enabled the bulk of the material in which all definite structure is lost, to be regarded in the light of the well-preserved specimen. Interesting as the distribution in the rocks of the imperfectly preserved fragments of wood is, they do not, with a few exceptions, call for detailed description. A few specimens show branching. One specimen of a different type affords evidence of the existence of a stem closely clothed with small, simple leaves.

Obscure plant-remains have been known for a considerable time from Nare Head and Polnare Cove on the east side of the Lizard and from Baulk Head on the west side. The strata in which these occur are mapped as Lower Devonian and constitute the Manaccan Beds of the local succession as given in the Survey Memoir (3). At Polnare they include branched specimens (Pl. XVI, Fig. 36). One specimen from Baulk Head has been described and figured as *Nematophycus Hicksii*? (4) This specimen is in the collection of the Penzance Museum, but I have not been able to obtain it for re-examination. In the light of the facts recorded in this paper, its provisional identification as *Nematophyton* is improbable and cannot be accepted unless confirmed by critical evidence (cf. p. 676, below). The

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existence of plant-remains in rocks of the Veryan, Portscatho, and Falmouth series has only recently been discovered by Miss E. M. Lind Hendriks, B.Sc. No such fossils had been previously known from these beds with the exception of a record of 'small organic fragments too obscure for identification' from a quarry in Portscatho beds south-west of Millewarn (3).

I am indebted to Miss Hendriks for sending me for investigation the plant-remains she collected at various localities. I am further indebted to the Director of the Geological Survey for sending me preparations made from the fragments that were first found by Miss Hendriks, and also the specimens previously collected by Mr. Pringle from the Manaccan Beds. I have visited the exposures on the east side of the Lizard along with Miss Hendriks and have been able to examine the occurrence of the plants and to collect material.

The stratigraphical succession and geological age of the clay-slates or 'killas' of this region, extending from Pendower, east of Falmouth to Gunwalloe and Porthleven on the west side of the Lizard, are at present being re-investigated by Miss Hendriks. This will doubtless lead to a revision of the classification of the beds that is given in the memoirs and maps of the Geological Survey dealing with this area. While recognizing the necessity and imminence of such a revision, the various localities in which fossil plant-remains have been found may be grouped here according to the sequence of the beds given in the Survey Memoirs.

Manaccan Beds.	Nare Head and Polnare Cove; Baulk Head; Jangye Ryn; Castle Hill.
Veryan Beds.	Pollurian Cove; Poldhu Cove; Gunwalloe Church Cove, Porthallow.
Portscatho Beds.	Millewarn Quarry; Porthcuel; Towan Beach; Portscatho Harbour; Porthcormick; Pendower.
Falmouth Beds.	Gunwalloe Fishing Cove; Cliff S. of Löe Bar.

In the Survey Memoirs the Manaccan Beds are regarded, without hesitation but on indirect comparative evidence, as of Lower Devonian age. The age of the rocks of the Veryan Series is given as Ordovician, while the Portscatho, Falmouth, and Mylor series are placed in descending order below the Veryan beds and their age given as Ordovician (?).

These beds are differently classified, and a different age assigned to them, by Upfield Green and Sherborn (6, 7). The arrangement of these geologists may be given in tabular form for comparison.

Falmouth.

Portscatho. (includes beds called Mylor, Grampound, Ladock, and Manaccan).

Veryan.

These are all placed as of Lower Devonian (Gedinnien) age. The Meadfoot Beds are regarded as Taunusian and the Staddon Beds as Coblenzian. On this view therefore all the plant-remains under consideration here would come from a low horizon of the Lower Devonian.

It will be evident that were the Lower Devonian age of these beds established the plant-remains they contain would be of great interest and importance, and this would be still more so were the beds shown to be pre-Devonian. On the other hand the nature of the plant-remains must be treated as an index of the age of the beds unless the age can be based on other evidence satisfactory to critical geologists. All that can be done at present is to give an account of the plant-remains, to refer them to their localities and their positions in the local succession, and to discuss our present knowledge of the occurrence of such plants in palaeozoic strata.

The wide distribution of obscure plant-remains in the clay-slates of this area of South Cornwall is shown by the list of localities given above, and their apparent vertical extension by their occurrence in beds that are classed in the Manaccan, Veryan, Portscatho, and Falmouth Series. As a rule the plants are represented by much altered and flattened fragments, the structural features of which are almost or quite lost. A general idea of their appearance is afforded by Pl. XV, Fig. 14, which represents a small portion of the split surface of the rock at Gunwalloe Church Cove. Much smaller fragments (cf. Pl. XV, Fig. 20) and also much larger specimens (Pl. XVI, Fig. 32) are also found. It will appear from this paper that there is reason to regard most or all of the ill-preserved specimens as indicating the distribution and abundance of fragments of fossil wood. It would be impossible to decide this were it not for the few specimens, to be described in detail later, that have afforded definite evidence. In the case of the majority of the fragments the structure is either wholly lost, or a longitudinal striation based on longitudinally running tubular elements is visible on examination by reflected light. An exceptionally good example of this is shown in Pl. XV, Figs. 15, 16. Such specimens do not usually provide evidence to identify them with certainty as wood, though their appearance suggests this. The carbonaceous material of the walls of the tubular elements has broken down into fragments and neither by reflected light, nor when the fossil is transferred to a cellulose film (cf. Pl. XV, Fig. 19) can the true structure of the wall be seen. Many such transfers were made. That shown in Pl. XV, Fig. 19, came from the counterpart of the specimen represented in Pl. XV, Fig. 18, and is in some ways exceptionally good. On the right-hand side the usual appearance of the tubular elements is visible, but those on the left-hand side further show the oblique end walls, indicating that the elements are tracheides and not continuous tubes. It is unnecessary to dwell further on the appearances presented by these altered pieces of wood. It may, however, be mentioned that the cavities of the

elements are often filled with reddish or orange granules of iron-oxide and that these contents, along with the black remains of the walls, give such appearances of structure as persist.

Hardly anything has been learnt of the external morphology of the plant or plants from which these fragments of wood were derived. Occasionally small branched specimens are found. Two of the best, collected by Miss Hendriks in the Veryan beds at Poldhu Cove, are represented in Pl. XV, Figs. 17, 18. That in Fig. 17 is enlarged six times and only measured 5 mm. in length; it shows the bases of three alternating branches or appendages. The somewhat larger specimen enlarged three diameters in Pl. XV, Fig. 18, has only one lateral branch.

None of the branched specimens met with in the other beds are as large or as highly ramified as some of those collected from Manaccan beds at Polnare Cove by Mr. Pringle. The remains are there preserved as graphitic incrustations in a dark rock. One of the best of these branched specimens is represented of natural size in Pl. XVI, Fig. 36. It not only shows two branches but one of these is again branched. Other fragmentary plant-remains are crowded in the rock. Little has been ascertained regarding the structure of specimens from Manaccan beds, either from Polnare or from the West side of the Lizard (Baulk Head, &c.). All that can be said is that many of the fragments show the tubular structure described above as characterizing ill-preserved fragments of wood. To this extent at least they connect with the plant-remains of the Veryan, Portscatho, and Falmouth beds.

The presence of these plant-fragments is a characterisic feature of all these rocks. Their uniformity of type, abundance, and mode of occurrence may have bearings on the conditions of deposition of the clay-slates. In much of the rock plant-remains are, of course, not evident. On the other hand they do not appear to be limited to particular layers although they are particularly abundant at places. At two widely separated localities they had given rise to thin layers of coal. One of the coaly layers is in the Veryan beds at Gunwalloe Church Cove and the other in the Portscatho Beds at Pendower.

The best specimen, showing all the main details of *Dadoxylon*, was found in immediate continuity with the coal layer at Pendower and indications of the passage of wood with the same structure into the coal, were observed. This specimen will first be described; an account will then be given of a few other specimens with less perfect but recognizable structural features. These serve to connect the bulk of the imperfectly preserved specimens with the one well-preserved example.

The specimen from Pendower is represented of natural size in Pl. XV, Fig. 1, as it appeared before any portion had been removed for the study of the structure. It lies on a piece of the thin coal layer already referred to.

The specimen itself is a piece of partially silicified wood, that appears to have broken or cracked across during decay and before petrifaction. It measured some 4 cm. in length by about 1·5 cm. across and was about 4 cm. thick. The piece of wood was oval in cross section but proved to be entirely composed of secondary xylem.

On examining the surface by reflected light it was evident that the structure was much better preserved than in any other specimen that had been met with. Very small fragments displaced from the surface with a needle and mounted in balsam were sufficiently thin and translucent to show various tissue-elements. Some were elongated and tubular and from the markings on the carbonaceous walls were evidently tracheidal (Pl. XV, Fig. 2). Others were parenchymatous, the rectangular brick-shaped cells showing that they were derived from medullary rays (Pl. XV, Fig. 3). The fragment figured is of interest because there is a suggestion of preservation of the cell-contents. The figure shows a round dark body in several of the cells that gives the appearance of a nucleus, besides more obscure indications of what may be other cell-contents. While the figure shows the appearance, it would be unwise to stress the possible interpretation. In many other cases the cell-cavity was occupied by a contracted black mass. The fact that such fragments could be readily detached showed the partial nature of the petrifaction and the softness of the resultant material as compared with thoroughly silicified wood.

The specimen seemed too soft to promise much success in cutting and grinding sections in the usual way. Another method of studying the structure was therefore adopted. A small piece of the petrified wood was split off from the portions that are seen in Pl. XV, Fig. 1, to be separated by the transverse cracks. The piece thus removed was flat, about 5 mm. across, 2·5 mm. long and some 2 mm. thick. It was placed in hydrofluoric acid to remove the silica. As a result it broke up into fragments, some of which, however, were of considerable size and retained the original thickness. They had, of course, become very delicate and friable on account of the removal of the silica, their cohesion now depending on the connexion of the carbonaceous material. The larger fragments were, with great care in the manipulation, washed free of hydrofluoric acid, transferred to absolute alcohol, then to chloroform and embedded in paraffin. They were then cut into serial sections on the microtome. The lengths of the paraffin ribbon were floated out on water on slides thinly coated with albumen fixative and, after draining off the water, were allowed to dry thoroughly. The paraffin was melted by placing the slide on the hot-plate and was dissolved off with xylol. The sections, without further treatment, were mounted in Canada balsam in the same way as any slide of serial sections.

From one of the fragments carefully orientated on the holder a series of accurately transverse sections was cut (Pl. XV, Fig. 4). When several

slides of these had been obtained the block was readjusted on the holder and cut longitudinally along its edge; this gave a number of slides of tangential-longitudinal sections (Pl. XV, Fig. 6). When the block was again adjusted so that sections were cut parallel to the flat surface of the fragment, radial longitudinal sections were obtained (Pl. XV, Fig. 5). Thus from a piece of wood measuring about 3 mm. in length, 2 mm. across, and slightly over 1 mm. in thickness numerous accurately transverse, tangential, and radial sections of a thickness of 6 μ were obtained. Other fragments were dealt with in the same way.

I am indebted to my laboratory assistant, Mr. E. Ashby, for the skilful work of obtaining these sections as well as for his help in other ways in the preparation and photographic illustration of this paper.

That the fragments cut into serial sections were fair samples of the wood was shown by grinding down one end of the specimen in Pl. XV, Fig. 1, until a complete cross-section of the piece of wood lying in the upper portion of the coal was exposed. The surface was polished, etched with hydrofluoric acid, coated with a solution of cellulose-acetate in amyl-acetate, dried, and the cellulose film stripped off. By this method, recently devised and described by Mr. Walton (16), complete transverse sections were obtained. These showed that the preservation of the wood was unequal; at places the structure had broken down completely but for the most part it resembled that of the fragments investigated. These proved to be samples of the best preserved tissue, their retention of consistency and form after treatment with hydrofluoric acid being doubtless due to this. The larger area of section obtained by the film-pull method was useful as showing the absence of any distinction of annual rings. The structure of this secondary wood has been almost entirely ascertained from the microtome sections.

Portions of transverse sections are shown in Pl. XV, Figs. 4 and 9. The tracheides, which are arranged in regular radial rows, are seen to be rectangular with their greater extension in the radial direction. While at places they are crushed and collapsed, their true form is preserved over most of the section. The organic substance of the middle region of all the walls appears to have been lost by decay, the carbonaceous material being in the position of the innermost or tertiary layer around the lumen. At favourable spots (Pl. XV, Fig. 9) a difference between the radial and tangential walls is recognizable, the former showing two or three pits in the substance of the wall. Little can be learnt, however, as to the detailed structure of the pits from the sectional view of walls in this state of preservation. The medullary rays are not very prominent but can be traced by their narrower cells elongated in the radial direction and sometimes by the dark carbonized contents of the cells. The contents are not prominent in any of the rays in the portions of transverse sections figured. Even in these small portions the rays do not extend across the section, a feature that

finds its explanation in their obliquity (cf. Pl. XV, Fig. 5). No parenchymatous elements other than the medullary ray cells are present in the wood.

A considerable portion of a longitudinal section in the tangential plane is shown in Pl. XV, Fig. 6 and smaller portions of similar sections are more highly magnified in Pl. XV, Figs. 7, 8. The radial walls of the tracheides, here seen in section, present unmistakable evidence throughout their length of bordered pits. As was the case for the transverse sections, however, no one pit can be found to show the complete construction owing to the condition of partial decay of the walls. Where the tangential longitudinal walls are seen in surface view the tracheide appears as if more or less filled up by a membrane that does not show any pitting; the tangential walls thus contrast with the radial walls as the latter are seen in the radial sections. The medullary rays are clearly shown by the tangential sections to be only one cell broad and usually two to four cells deep, though rays of six cells and others of only one cell have been noticed. As is well shown by the ray to the right in Pl. XV, Fig. 7, dark masses, representing the contracted contents are often present in the cells.

A considerable extent of a radial section including a medullary ray is represented in Pl. XV, Fig. 5, while further details are illustrated at higher magnifications in Pl. XV, Figs. 10–13. The oblique position of the medullary rays relatively to the longitudinal axis of the tracheides, which is well shown in Pl. XV, Figs. 5 and 10, is a very general and peculiar feature of this piece of wood. It explains the impossibility of following any one ray far in the transverse sections. Where the tangential walls of the tracheides appear in section the clear median space has on either side the continuous inner layer of the two adjoining tracheides, the absence of pits leading to a quite different appearance from that presented by the radial walls in section. This is shown by a comparison of the radial sections in Pl. XV, Figs. 5 and 10, with the tangential sections in Pl. XV, Figs. 6 and 8. The radial walls, seen in surface view in Pl. XV, Fig. 5, appear to have been uniformly pitted over their whole extent; any differences are readily accounted for by the more or less perfect state of preservation. In considering them the fact that the two films belonging to the complete wall between two tracheides may have become separated or not be both included in the section must be borne in mind. The most perfect and undisturbed condition is shown in the wall of a tracheide represented at a magnification of 400 diameters in Pl. XV, Fig. 13. Here the wall is completely covered by typical crossed pits. These form three rows and it is clear that the obliquely placed oval pores which cross one another belong to the two layers of wall belonging respectively to the two adjacent tracheides. Where the openings of the two oval pores coincide in the central region there is the appearance of a small clear area, but this is, of course, not a circular

pore. The more common appearance of a set of obliquely inclined oval pores all sloping in the one direction (cf. the upper part of Pl. XV, Fig. 10) is given when only the layer of wall belonging to one tracheide is seen. The single and the double layer, with, in the latter case, the appearance of crossed pits, are shown in different portions of the tracheide on the left in Pl. XV, Fig. 11. In many cases another appearance that is shown in Pl. XV, Fig. 12, is met with. Here in the area of the wall corresponding to a double pit in Pl. XV, Fig. 13, a dark, circular or discoid structure with what appears to be a small circular pore is seen. These structures at first sight suggest circular bordered pits with a round pore but there is no reason to doubt that (as has been clearly explained by Gothan (5)) they are casts of the pit-cavity of such pits as those more truly seen in Pl. XV, Fig. 13. The readiness with which the pit-cast becomes slightly displaced confirms this and is indicated in Pl. XV, Fig. 12.

It has been mentioned that the medullary rays in this piece of wood are typically oblique to the long axis of the tracheides. Their radially extended, brick-shaped cells, which form one to five rows, have thin walls. These and the contents suffer more from the removal of the mineral matter than do the tracheide walls, but their outlines are still clear in the sections. The radial extent of the cells of the ray is as a rule about equal to the radial width of two or three tracheides. The pitted areas of adjacent tracheides where they cross the ray cell, have not been very clear in any case, but there is nothing to suggest that the pitting of these 'fields' differs markedly either in number or shape from that of a similar area of one half the thickness of a tracheide wall. This point is not illustrated in detail, but the walls in question are seen in Pl. XV, Fig. 5, at the extreme right of the ray and Pl. XV, Fig. 10, at the extreme left.

No information has been obtained as to the length of the tracheides. Their width is regularly greater in the radial than in the tangential direction, and there is some variety in the size of different elements. On the average, however, the radial width appears to be about 45 μ and the tangential width about 35 μ. The diameter of the bordered pits is about 12 μ.

The wood described and illustrated above has the general construction typical of *Dadoxylon*. Without entering here into a differential comparison with other specific types of the genus it seems advisable, if only for stratigraphical reasons, to distinguish this material of secondary wood of Cordaitalean type by a specific name. I therefore propose to call it *Dadoxylon Hendriksi* after the discoverer. It is characterized by the uniform distribution over all the radial walls of the tracheides of crossed pits in three (occasionally four) rows, and by the obliquity of the medullary rays.

While no other specimen has yet been found that has the structure as perfectly preserved as that from Pendower described above, some others

show in various ways indications of corresponding structure. They may be referred to the same species and justify the inclusion of other specimens in which the structure has become obscured or lost.

The specimen which is represented, enlarged two diameters, in Pl. XVI, Fig. 27, was found by Miss Hendriks in beds that are mapped as belonging to the Falmouth Series. Small fragments showing obscure tubular structure had previously been noticed in the rocks at Gunwalloe Fishing Cove, but this and a few other specimens came from the cliffs further west, about 500 yards to the south of Löe Bar. It was a flattened piece of wood and was highly pyritized, being in great part of a golden yellow colour. Cracks across the vegetable material were filled in with white crystalline mineral and portions of the surface between the cracks were brown and showed by reflected light remains of the structure of the wood. A portion of the surface photographed by reflected light is shown in Pl. XVI, Fig. 28. Not only can the walls of the longitudinally running tracheides be followed, but a medullary ray can be traced for some distance at the upper right-hand side, and another less clearly lower in the figure. The better preserved ray is some five cells deep and like the other is slightly oblique. It was not possible to draw any safe conclusions as to the markings on the tracheide walls from examination with reflected light, but the features mentioned were sufficient to show that this was a piece of wood of similarly high organization to that found in the Portscatho Beds at Pendower.

Since the study of the pyritized specimen could not be carried further by sections either prepared by grinding or microtoming, another method had to be adopted. This was based on Mr. Walton's use of cellulose films referred to above (p. 668). It was found by preliminary experiments on suitable woody incrustations that film-pulls could be obtained from them either with or without previous treatment with hydrofluoric acid. The surface of such specimens is, of course, irregular, but in favourable cases isolated elements or translucent films of tissue were thus obtained from incrustations. By the use of this modification of the method several complete film-pulls were taken from the specimen shown in Pl. XVI, Fig. 27, some with and some without preliminary etching. While the pyritized material, even in the thin layer thus removed from the portions with structure, was very opaque, definite information was obtained from a few favourable areas of the films.

Two such regions are photographed in Pl. XVI, Figs. 29 and 30. Both show portions of tracheides which afford information as to the pattern on the radial walls. These figures are at the same magnification as Pl. XV, Figs. 10 and 11, of the Pendower specimen and are directly comparable. The obliquely placed pits shown in Pl. XVI, Fig. 29, resemble those in the upper part of Pl. XV, Fig. 10. Further, just as the appearance of circular

pits was given by the discoid casts seen in some of the tracheides of Pl. XV, Fig. 10, and more highly magnified in Pl. XV, Fig. 12, the pyritized specimen revealed opaque discoid casts, two or three to the breadth of a tracheide. These are shown in several of the tracheides of the area photographed in Pl. XVI, Fig. 30. Portions of medullary rays were also observed in the film-pulls. The resemblances in these various details are so close that this specimen may reasonably be identified with *Dadoxylon Hendriksi*.

Associated with this specimen were a number of small black incrustations suggestive of pieces of wood only slightly flattened. While these showed no details of structure, enough could be made out by reflected light to justify regarding them as obscurely preserved or altered pyritized remains of what may naturally be presumed to be the same type of wood. This conclusion is of some importance for it extends to similar pyritized black incrustations met with elsewhere in the clay-slates. They are known from Baulk Head and from Gunwalloe Church Cove but the most striking were found at Portscatho harbour. The latter included a linear black structure of oval cross-section measuring 25 cm. × 1 cm. In the light of the other pyritized specimens there is little doubt that this was a woody stem, though nothing remains of the structure except some pyritized casts of tubular elements.

Some incrustations from Porthcuel afford further evidence connecting the imperfectly preserved specimens with the type of secondary wood with medullary rays. They were much flattened, but fairly large, measuring some 3 cm. by 1·5 cm. Portions of the most interesting specimen are illustrated by the photographs in Pl. XV, Figs. 15 and 16, and Pl. XVI, Fig. 31. It agrees with many others from various localities in showing parallel, tubular elements, the walls of which are represented by granular carbonaceous material while the cavities are filled with reddish or yellow iron oxide. The incrustation was surrounded by an incomplete layer of white mineral, through openings in which the tubular construction is clearly visible under reflected light (Pl. XV, Figs. 15 and 16). The feature of special interest in this specimen is that it further demonstrates the presence of medullary rays. These are evident mainly owing to the shape of the cells being preserved by the yellow granular material that fills them. The portions of rays several cells deep are, however, unmistakable as they cross the tubular elements. The appearance of such a region is represented in Pl. XVI, Fig. 31, and shows one ray about five cells deep, while another is present further to the left at the bottom edge of the figure.

None of the specimens described above as showing definite evidence of being wood have come from Veryan Beds.[1] Flattened incrustations with more or less evident remains of tubular construction are abundant in these

[1] Since this paper was completed a well-preserved piece of pyritized wood referable to *Dadoxylon Hendriksi* has been found at Porthallow in beds mapped as Veryan.

rocks especially at Gunwalloe Church Cove (cf. Pl. XV, Fig. 14) but numerous attempts to discover traces of more definite structure were for long unsuccessful. Among these incrustations are some of large size in which the material of the plant is represented by a flattened carbonaceous mass. A portion of such a specimen measuring about 3 cm. across is shown of natural size in Pl. XVI, Fig. 32. The carbonaceous material sometimes exhibited tubular construction under reflected light, and in such cases some remains of structure were found, when a piece was impregnated with synthetic resin and then ground down to give a longitudinal or transverse section (Pl. XVI, Fig. 33). In no case, however, did the walls of the tubular elements show distinctive markings. Film-pulls of the remains of the tissue in contact with the rock gave better results. As Pl. XVI, Fig. 34 (which is of such a preparation from the specimen in Pl. XVI, Fig. 32), shows, the tissue has the general appearance of wood; but here also the walls of the elements have lost the original pattern. One fortunate film-pull from another similar specimen, however, showed a few elements with the structure of their walls sufficiently preserved to provide critical evidence (Pl. XVI, Fig. 35). Two of the tracheides in this figure show the oblique slits of the pits. This figure is comparable with Pl. XV, Figs. 10 and 11, and Figs. 29 and 30.

The definite traces of pitted tracheides or of medullary rays in the three specimens last described serve to connect the bulk of the more imperfect incrustations with the well-preserved secondary wood of the Pendower specimen of *Dadoxylon Hendriksi*. They further afford evidence of the existence of wood of this type in rocks of the Falmouth, Portscatho, and Veryan series. No definite structural evidence has as yet been obtained from remains in the Manaccan beds, though some of them closely resemble the imperfectly preserved remains from the other beds and were probably of the same nature. It would appear that most of the plant-remains were derived from fragments of secondary wood and afford no information as to the external form of the plant. The few branched specimens of small size are an exception, and (if from the same plant) might be expected to afford information as to the primary structure. But they have all proved to be imperfectly preserved, and nothing can be said as to the primary xylem.

Brief mention must be made of the first organic remains discovered by Miss Hendriks in these rocks. They were some very small flattened incrustations in the clay-slates at Portscatho and Porthcuel and were at first supposed to be fish-remains. Preparations showing the partially preserved tissues in longitudinal and transverse section had been skilfully prepared at the Geological Survey Museum and were sent to me for examination. The appearance of one of the small pieces in longitudinal section is shown in Pl. XV, Fig, 20, and of a portion of another in transverse section in Pl. XV, Fig. 21. The tissue is uniform throughout, the elements

appearing as longitudinally running parallel tubes. Careful search only revealed doubtful indications of occasional cross walls. The structure is revealed by the contents rather than by the walls, which are clear with only slight remains of carbonaceous material (Pl. XV, Fig. 21). It is doubtful to what extent this tissue has been altered and what was its original appearance. Miss Hendriks's further discoveries render a full account of these fragments unnecessary, but one interesting point must be mentioned.

In the longitudinal section of one small and badly decayed specimen (Survey Slide, E. 13165) some elements were present which appeared to have a spiral or annular thickening of their walls. These were originally noticed by Professor Gordon who appropriately compared them with the tracheides of *Asteroxylon Mackiei*. A portion of the section showing these thickened elements is represented in Pl. XV, Fig. 22. It was, and still is, difficult to interpret this isolated preparation, though subsequent discoveries have confirmed Professor Gordon's recognition of it as affording evidence of wood. The thickening is peculiar; the projections of the wall into the lumen are narrow, the broader bands in Pl. XV, Fig. 22, being the intervals between them shown by the material filling the lumen. In this feature there is remarkable agreement with the tracheides of *Asteroxylon Mackiei*. No quite similar specimen has been met with, but among numerous transfers made from the small carbonaceous fragments exposed by splitting the rock one was found that showed considerable agreement. The portion of this transfer represented in Pl. XV, Fig. 23, shows the carbonaceous outline of some tissue-elements to the right and to the left, some elements the walls of which are represented by narrow carbonaceous bars. At places these are united so as to suggest a pitted wall. While these structural features require to be placed on record, their interpretation must await the discovery of more completely preserved specimens.

The last specimen to be described, which is also at present unique and problematical, is represented of natural size in Pl. XV, Fig. 24, and enlarged two diameters in Pl. XV, Fig. 25. It was found by Miss Hendriks in the Veryan beds on the south side of Poldhu Cove. It is a slightly convex cast of a cylindrical axis about 5 mm. in width and 2 cm. long. The concave counterpart, which is a little longer, was also collected. The surface of the cast is marked by numerous small depressions arranged in a complicated spiral (Pl. XV, Fig. 25). The black opaque organic material which forms an incomplete thin layer over the surface is most marked in these depressions. It was also present on the concave surface of the counterpart. A transfer was made from a small portion of the latter but nothing could be ascertained as to the histological structure.

Later a film-pull of the counterpart revealed the presence of a curved, linear, pointed structure lying on the matrix close to the specimen. This detached carbonaceous structure is 2 mm. long and is highly suggestive of

one of the small leaves that were probably borne on this stem. The whole appearance of the specimen justifies the conclusion that it was part of a plant the axis of which bore spirally arranged small appendages. While nothing certain can be said as to its affinities the points of resemblance to *Thursophyton* or *Asteroxylon* are of interest.

Concluding Remarks.

The difficulty of estimating the significance of the fossil plants discovered by Miss Hendriks in the clay-slates of South Cornwall is due to the uncertainty as to the geological age of these strata. If it were justifiable to assume as established even the Lower Devonian age of the beds it would only be necessary to point out the importance of the fossils as affording the earliest record of plants with such complex structure. Since the geology of the district is being re-investigated the age of the beds must be regarded as an open question in these remarks. It is thus necessary to consider the possible bearings of the palaeobotanical discoveries on this question.

The general distribution of the plant-remains in the rocks of a somewhat extensive area in South Cornwall is of interest, especially when taken with the absence or rarity of animal remains. The distribution of the multitudinous fragments of wood is especially significant and suggests comparison with the mode of occurrence of fragments of *Nematophyton* in the Downtonian of England and Wales. A solution of the geological problem thus presented as to the place and mode of deposition of the sediments is desirable.

The uniformity in general type of the altered plant-remains in rocks that have been divided into the Manaccan, Veryan, Portscatho, and Falmouth series is remarkable. It would appear to point to attributing little importance to these stratigraphical distinctions as indicating any considerable differences in geological age. This is more in accord with Upfield Green and Sherborn's views (6, 7) as to the association of the various beds than with the views expressed in the Survey Memoirs. This consideration raises another geological problem which is outside the present discussion although it has important bearings upon it.

The plant-remains described in this paper are certainly of two kinds. They may, of course, be derived from more numerous kinds of plants but nothing certain can be said as to this. There are (*a*) the slender axis that probably bore small spirally arranged appendages, and (*b*) the larger or smaller pieces of fossil wood. Most of the latter may be reasonably assumed to be portions of *Dadoxylon Hendriksi*, since no other type than this has been demonstrated by means of structural details. The nature of the more slender branched specimens from the Veryan beds and also those from the Manaccan beds remains doubtful.

The small axis with spirally arranged markings suggesting the places of insertion of small leaves that must have closely clothed the stem suggests comparison with the type of *Thursophyton* and *Asteroxylon*. Identification is out of the question until more details can be ascertained than is possible from the single specimen so far met with. The comparison with *Asteroxylon* receives indirect support from the resemblances of the tracheidal thickening in a fragment from Porthcuel to that characteristic of *Asteroxylon Mackiei*. These remains, so far as they go, are in favour of attributing a Middle Devonian age to the rocks.

The great majority of specimens are those with more or less distinct indications of striated structure based on longitudinally running tubular elements. The microscopic study of such imperfectly preserved material at first suggested that the plant might be *Nematophyton*. As has been mentioned, the mode of occurrence of the fragments in the rock is very similar to that of *Nematophyton* in Downtonian strata, and the tubular construction evident by reflected light and in transfers and sections (cf. Pl. XVI, Fig. 33), appeared consistent with this. In no case, however, could evidence be obtained of the existence of the second system of narrow tubes characteristic of *Nematophyton*, and it was therefore not possible to refer the remains to this plant. The subsequent discovery of a specimen showing the structure of secondary wood of *Dadoxylon* type and of specimens linking the ill-preserved flattened incrustations to this have shown that caution was fully justified. There is no critical evidence that any of the remains are *Nematophyton*, and it is improbable that any of them are derived from that type of plant.

The outstanding and fully established facts are that a typical *Dadoxylon* occurs in strata referable to the Portscatho beds and that the best preserved remains prove the existence of this plant in the Falmouth and Veryan beds also. It is almost certain that the ill-preserved remains in the Falmouth, Portscatho, and Veryan beds (and probably in the Manaccan beds also) indicate a wide distribution and abundance of this type of plant. It is the significance to be attached to the discovery of *Dadoxylon Hendriksi* in these rocks that has therefore to be considered.

The known occurrence of secondary wood of this or similar type in the older palaeozoic rocks of the British Isles and elsewhere is as follows: Such wood is, of course, well known from Carboniferous rocks. A *Dadoxylon* from the 'Penlan Quartzite' near Kidwelly was at first referred to the Upper Devonian but is now, on the evidence of the associated remains and on lithological grounds regarded as of Lower Carboniferous age (2). So far as I know, no specimen of *Dadoxylon* has been described from the Upper Devonian or Upper Old Red Sandstone of Great Britain and Ireland. There is no reason to doubt that plants with this type of secondary wood existed at the time, for a number of species are known from the Upper

Devonian rocks of North America (1, 14). Some of these are of the usual type, but others have the pits limited to certain regions of the radial walls and are placed in the genus *Callixylon*, the type species of which is *Callixylon Trifiliewi* from the Upper Devonian of Russia (17).

There is some uncertainty as to whether some North American specimens of *Dadoxylon* come from the Upper Devonian or are really as is stated of Middle Devonian age. But, apart from those of doubtful age, it seems clear that the type is known from Middle Devonian rocks there (1, 14). In the Middle Old Red Sandstone of Scotland pieces of what are certainly secondary wood occur as black incrustations, sometimes of large size. The records of *Dadoxylon* (or *Araucarioxylon*) from these rocks (13) are based on such specimens, but there is no critical evidence of the nature of the tracheides or their pitting ; the application of these names is thus not at present justified. An early account and figure (15) showing the pitting in such a specimen has not been confirmed, and I have been unable to trace the preparations. It is, however, not improbable that some of these incrustations will prove to be really of the type of *Dadoxylon*, but this has so far not been demonstrated. In the case of a number of specimens I have been able to learn something of the structure by means of film-pulls. In all so far seen, however, the tracheides were more like those of *Palaeopitys Milleri* which is known fully in the petrified condition (9). The secondary wood of this plant is complex, but quite distinct in type from that of *Dadoxylon*, having pits, with transversely extended pores, on all the walls of the tracheides. The secondary wood of *Aneurophyton* from the Middle Devonian of Germany is similar (11). A specimen of secondary wood from the Middle Devonian of North Devon (Ilfracombe Beds) that is in the Geological Survey Museum has also been shown by film-pulls to differ from *Dadoxylon*. There is ample proof of the presence and even the abundance of pieces of secondary wood of large size in the Middle Devonian period, but only in a few cases has the wood been shown to have the characters of *Dadoxylon*.

No secondary wood is known on adequate evidence from the Lower Devonian or Lower Old Red Sandstone of Great Britain or elsewhere. It is, of course, quite possible that plants with secondary xylem existed at this period, and the primary vascular system of a few plants has been demonstrated. The large incrustations on which records of secondary wood from the Lower Old Red Sandstone have sometimes been based have proved in the cases that have been critically examined to be pieces of *Nematophyton*. The same applies to the Downtonian rocks in which fragments of *Nematophyton* are abundant at places.

We have practically no knowledge of the land-plants of Silurian and Ordovician times. In the case of most of the few records there are elements of doubt, either as to the nature of the plant-remains or as to the age of

the rocks in which they are found. But highly organized plants resembling some of those known from the Middle Devonian, occur in Australian rocks that are regarded by the most authoritative geologists there as Silurian (12). There are some indications of land-plants from Ordovician rocks, but nothing is certainly known. Organic material with traces of apparently woody structure has been described as *Archaeoxylon Krasseri* even from Pre-Cambrian rocks (10).

The general result of the above survey of the records from Great Britain and other regions is that, on our present knowledge, wood of the type under consideration in this paper occurs in Upper Devonian rocks and rather more doubtfully in rocks of Middle Devonian age. It has not been shown to occur in Lower Devonian or earlier rocks.

The presence of *Dadoxylon Hendriksi* in the clay-slates of South Cornwall would by itself be consistent with the beds being Carboniferous or Upper Devonian, since such secondary wood is already known from rocks of these periods. It would also be consistent with the beds being determined as Middle Devonian, since it is clear that plants with considerable development of secondary wood and some with wood of *Dadoxylon* type existed at this earlier period. If this were shown to be the age of the beds the specimen from Pendower would be the best known wood with the structure of *Dadoxylon* from the Middle Devonian. If the Cornish beds are shown to be Lower Devonian (which is the youngest age that has yet been attributed to them by geologists) the interest of the *Dadoxylon* they contain would be very great, since it would constitute the earliest record of such a highly organized type of wood. The interest of the plant-remains would be extreme were the beds shown to be of earlier age than the Lower Devonian.

It would be unsafe to say that the occurrence of *Dadoxylon* in the Veryan, Portscatho, and Falmouth beds renders a Lower Devonian or even a still earlier age of these rocks out of the question. But it certainly does not support such a view, and, on our present knowledge, is in favour of a younger age. Clear evidence from animal fossils or stratigraphy would be required to warrant placing the rocks earlier than the Middle Devonian. Taking into consideration the small-leaved stem and the tracheides resembling those of *Asteroxylon*, the palaeobotanical evidence appears to indicate, but not to establish, a Middle Devonian age. The plant-remains from South Cornwall collected by Miss Hendriks, and especially the well-preserved specimen of *Dadoxylon*, are interesting and surprising discoveries in the rocks of this region. It becomes increasingly important for botanical as well as geological reasons to have the age of these beds determined with as great precision as possible.

Summary.

1. Plant-remains collected by Miss E. M. Lind Hendriks from the Falmouth, Portscatho, and Veryan Beds of South Cornwall are described. These beds have been variously regarded as of Ordovician or of Lower Devonian (Gedinnien) age. Plant-remains were previously known from beds classed as Manaccan and regarded as of Lower Devonian age.

2. Most of the remains are of larger or smaller fragments of what is probably in most cases secondary wood. A few branched specimens are described and compared with larger branched specimens from the Manaccan beds.

3. By adopting suitable methods the detailed structure of the secondary wood has been fully ascertained in one specimen from the Portscatho Beds at Pendower. This is named *Dadoxylon Hendriksi.* Traces of definite structure corresponding to that shown by this specimen have been found in other specimens from the Portscatho Beds, in a specimen from the Falmouth Beds, and in a specimen from the Veryan Beds. These various specimens link on the bulk of the ill-preserved remains to *Dadoxylon.*

4. Small fragments with altered structure, but in one case with indications of spirally thickened elements, are described from the Portscatho Beds.

5. A single specimen of a slender axis that bore small spirally arranged appendages, is described, from Veryan Beds.

6. The interest of these plant-remains in relation to the possible age of the beds is discussed. If the beds are shown by geologists to be older than the Upper Devonian and of Middle Devonian age, these plant-remains would afford one of the earliest satisfactory records of such highly organized secondary wood. The interest would be proportionately greater should the beds prove to be Lower Devonian or pre-Devonian.

7. It can be said that wood of this organization is at present known from the Upper Devonian and probably from the Middle Devonian; it is not known from Lower Devonian or Lower Old Red Sandstone rocks. It would, however, be unsafe to decide on the ground of the plant-remains alone that the Cornish beds under consideration must be younger than the Lower Devonian, though, taking into consideration all the plant-remains, a Middle Devonian age appears to be indicated.

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EXPLANATION OF PLATES XV and XVI.

Illustrating Professor W. H. Lang's paper On Fossil Wood (*Dadoxylon Hendriksi* n. sp.) and other Plant-remains from the Clay-Slates of South Cornwall.

(All the figures are from untouched photographs).

Figs. 4–13 are from microtome sections 6 μ in thickness.
The numbers of the specimens refer to the Geological Survey Collection.

PLATE XV.

Fig. 1. Specimen of *Dadoxylon Hendriksi*, n. sp. on surface of a piece of the layer of coal from Pendower. The secondary wood is divided into a number of portions by obliquely transverse splits. Nat. size. No. 48661.

Fig. 2. Fragment from the surface of the specimen in Fig. 1, showing three tracheides enclosed in the matrix. The carbonized pitted walls are more or less imperfect. × 100.

Fig. 3. Another fragment from the specimen, showing a few parenchymatous cells of a medullary ray. The cell contents, supported by the petrifying matrix, have a suggestive resemblance to a nucleated protoplast. × 250.

Fig. 4. Transverse section of the secondary wood. × 100.

Fig 5. Radial longitudinal section of the wood, showing the tangential walls of tracheides in section and, the radial walls in surface view. A medullary ray, three cells deep, crosses the tracheides obliquely. × 100.

Fig. 6. Tangential longitudinal section of the wood. × 100.

Figs. 7, 8. Tangential longitudinal sections, showing the pitted radial walls of the tracheides in section and medullary rays, two, hree, and four cells in height cut across. × 250.

Fig. 9. Transverse section of the wood, showing tracheides and medullary rays. × 250.

Fig. 10. Portion of a radial section, showing tracheides and medullary ray cells. The portions of tracheides above show the oblique pores of the pits of one layer of the wall; those below the ray show pits with circular casts. × 250.

Fig. 11. Portion of another radial section, showing tangential walls of tracheides in section, and radial walls with different states of preservation of the pits in surface view. × 250.

Fig. 12. Portion of another radial section to show the casts, giving the appearance of circular pits with a round pore. × 400.

Fig. 13. Tracheide from another radial section, showing the true form of the pits with crossed oval pores. The pits are in three rows and completely cover the radial wall. × 400.

Fig. 14. Flattened fragmentary incrustations in the rock from Gunwalloe Church Cove. Nat. size. No. 48662.

Fig. 15. Portion of flattened incrustation from Porthcuel, showing distinct tubular structure, representing the tracheides. × 5½. No. 48664.

Fig. 16. Portion of the specimen in Fig. 15. × 11.

Fig. 17. Small branched specimen from Poldhu Cove. × 6. No. 48666.

Fig. 18. Branched specimen from Poldhu Cove. × 3. No. 48667.

Fig. 19. Transfer of part of the counterpart of the specimen in Fig. 18, showing the tubular structure of the imperfectly preserved wood on the right, and the outlines of tracheides showing end walls on the left. × 50.

Fig. 20. Longitudinal section of one of the fragments first found in the clay-state at Porthcuel. × 25.

Fig. 21. Portion of transverse section of another fragment showing the tubes in cross section. × 250.

Fig. 22. Portion of longitudinal section of another fragment, showing appearance of annular or spiral thickening of some of the walls. × 250. Geological Survey Slide. No. E. 13165.

Fig. 23. Portion of transfer of a similar fragment, showing the outlines of the tubes or tracheides and annular or pitted thickening of the walls of some of the elements. × 200.

Fig. 24. Specimen from Poldhu Cove with probable indications of the position of small crowded spirally arranged appendages. Nat. size. No. 48668.

Fig. 25. Same specimen. × 2.

PLATE XVI.

Fig. 26. Film-pull of portion of the counterpart of the specimen shown in Fig. 24. On the right at *x* is a detached pointed leaf-like structure. × 4.

Fig. 27. Pyritized flattened incrustation with structure partially preserved from the Falmouth beds, 500 yds. south of Löe Bar. × 2. No. 48663.

Fig 28. Portion of the specimen in Fig. 27 photographed by reflected light to show the outlines of tracheides and portions of two medullary rays. × 52.

Fig. 29. Portion of a film-pull of the same specimen, showing tracheides with obliquely pitted walls. × 250.

Fig. 30. Another portion of a film-pull of the same specimen, showing tracheides with discoid pith-casts on the radial walls. × 250.

Fig. 31. Small portion of the specimen represented in Fig. 15 photographed to show a medullary ray crossing the tracheides. × 28.

Fig. 32. Portion of a large incrustation, in which the wood is represented by a carbonaceous mass, from the Veryan beds at Gunwalloe Church Cove. Nat. size. No. 48665.

Fig. 33. Small portion of a ground section of the carbonaceous material of the specimen in Fig. 32, showing tubular elements that have lost their definite markings. × 100.

Fig. 34. Portion of a film-pull of the rock enclosing the carbonaceous layer in Fig. 32. A general view of the tracheides, which have however lost their definite markings. × 100.

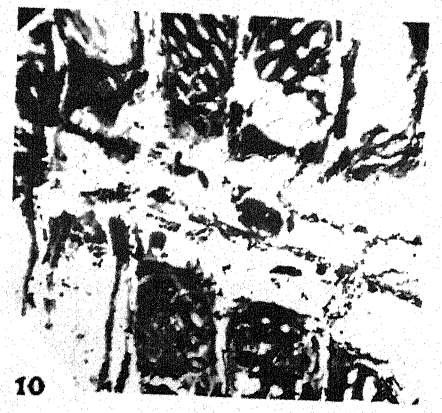
Fig. 35. Portion of a film-pull of a similar specimen from the same locality. It shows portions of the walls of two tracheides with obliquely placed pits. × 250.

Fig. 36. Specimen collected by Mr. Pringle from the Manaccan Beds at Polnare Cove. The dark rock bears numerous graphitic incrustations. These are mostly fragmentary, but one specimen shows two branches, one of which is again branched. Nat. size. No. Pr. 685.

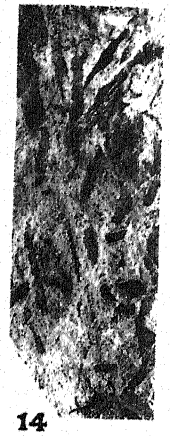

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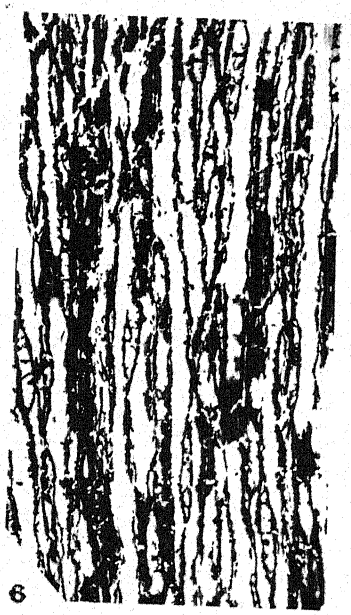

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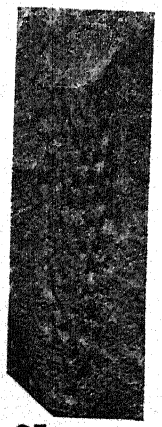

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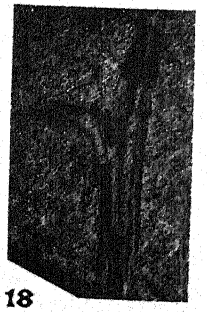

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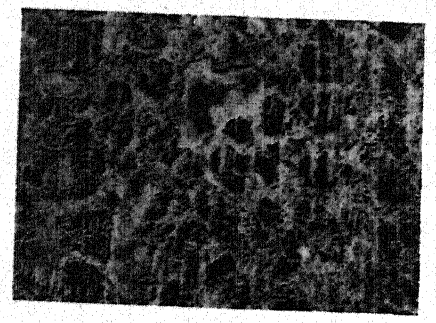

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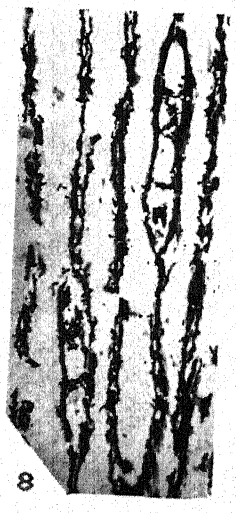
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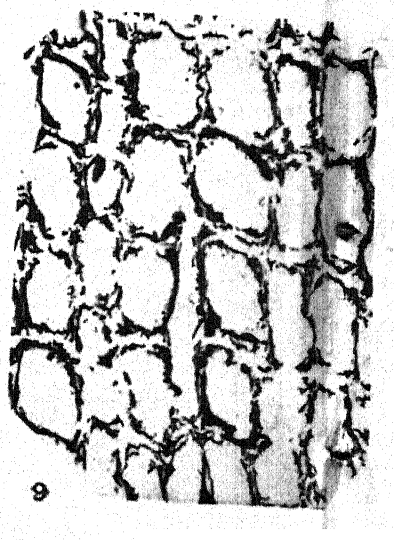

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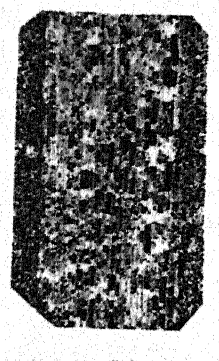

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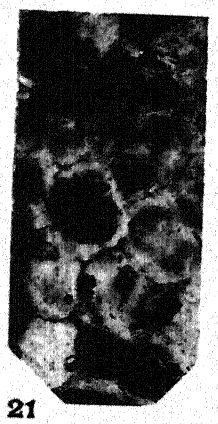

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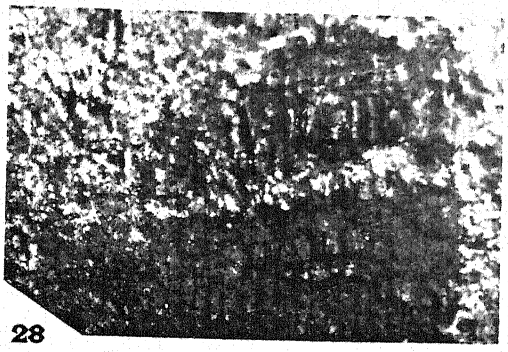
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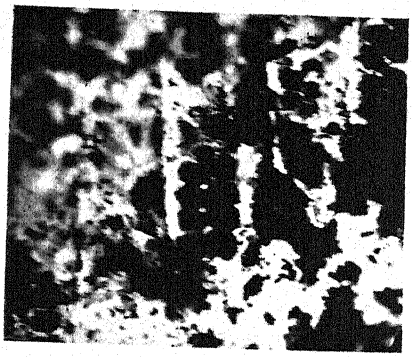
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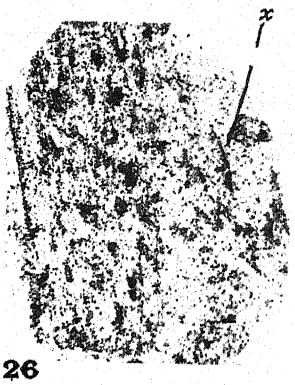



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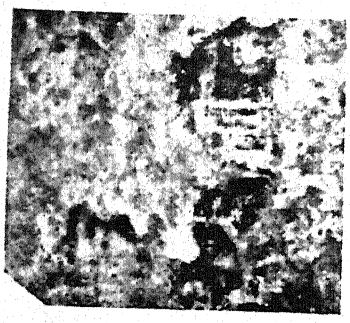

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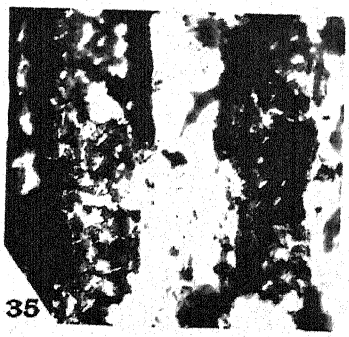

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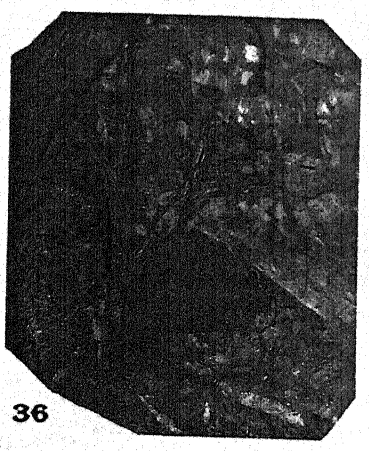

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Huth, London.

Experiments on the Production of Diseased Shoots by the Hop Downy Mildew, Pseudoperonospora Humuli (Miy. et Takah.), Wils.

BY

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With Plate XVII and six Figures in the Text.

I. INTRODUCTION.

IN the course of an attack by Downy Mildew (*Pseudoperonospora Humuli*) the hop plant (*Humulus Lupulus*), whether in the cultivated or wild state, frequently exhibits, in addition to other well-known symptoms, a serious stunting and deformity of the stem ('bine'). This injury is commonly shown by the young shoots which have only just emerged from the soil in spring; they may attain a height of about 25 cm., but they never succeed in further development, and eventually wither and die.[1] Internally the whole shoot is found to be invaded by mycelium, which passes from the stem into the petioles and laminae and forms, under moist weather conditions, a dense black mass of conidiophores and spores on the ventral surfaces of the leaves, in succession, from the oldest to the youngest. The mycelium in such cases usually extends to the base of the stem, and on some few occasions has been traced into the root-stock.

Similar deformity of the stem, with sudden and complete suppression of apical growth, occurs in the case of other bines which until then have succeeded in making normal and rapid development; it may take place when the stems have reached any height between 90 cm. and 450 cm., and its effects as a rule are evident externally over only a short length of the terminal part of the bine.[2] Internally the mycelium is found just behind the growing point, and usually extending through the whole of the stunted

[1] Such infected shoots, on account of their position, their short erect growth and diminutive leaves, have been designated 'basal spikes'.

[2] Apical parts of hop bines when thus stunted by Downy Mildew are known as 'terminal spikes'.

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part of the stem; sometimes it occurs also lower than this and may be found in nodes at which there are no external symptoms. When the mycelium is present in the stem below the visibly affected nodes and internodes constituting the 'spike', it is often discontinuous, and healthy nodes, internodes, or parts of internodes, may be found when tracing the course of the hyphae in a downward direction.

The lateral shoots which are produced at the nodes are also susceptible to attack. Their growth may be checked by the fungus as in the two previous instances, or their development from the bud may even be entirely prevented.[1] Both or only one of the laterals may be thus affected at any node or nodes on the stem. Internally, diseased laterals are permeated by mycelium which extends through the whole length of the stunted region; if the entire side-shoot is affected the mycelium can often be traced into the node of the main stem; here it may be localized or it may extend into the internodes, sometimes forming a continuous connexion with mycelium existing in the adjoining nodes above or below.

No statement exists in the early record from Japan (5) that *P. Humuli* causes any deformity or hypertrophy of the host plant. Similarly there is complete lack of information on this point in the published account of the occurrence of the disease on wild hops in America (3). On its arrival in Europe it was first observed at Wye, in the south-east of England, on cultivated hops in 1920 (17), but it was not until 1923 that deformity of the shoots was noticed on a considerable number of plants. During this and the following year (1924) the fungus became more widespread in England, and it was possible by observation of its activities in hop gardens to gain an impression—more or less complete—of the effect of Downy Mildew on the hop plant and on the practice of hop growing in this country. In the accounts published (6, 7, 8) the occurrence of deformed shoots ('spikes') and their importance as a means whereby the fungus is spread, was fully emphasized, but at the same time it was stated (7, p. 127) that 'the economic damage caused by hypertrophy of the shoots . . . has been negligible'.

It was not until the following season (1925), when 'spiked' growths appeared in hop gardens to such an extent that they deprived the grower of sufficient healthy bine on which to grow his hops, that their full importance as one of the worst features of the disease became recognized (9, 10, 11).

Investigations during that year provided information (18) as to the extent of internal invasion by the fungus, and led to certain hypotheses as to the way in which 'spiked' growths might be caused. During 1926 and 1927, although 'spikes' were always present, no such serious situation, threatening a shortage of bine, occurred; in 1928, however, a second occurrence of 'spikes' on a great scale, similar to that of 1925, was experienced in Kent.

[1] Diseased and stunted side-shoots constitute the third type and are known as 'lateral spikes'.

There was then no experimental evidence to indicate the way in which 'spiked' growths were formed, whether internally by mycelial invasion or externally by spore-infection. In the spring of 1928 the following experiments were carried out to obtain information on this point.

II. Experimental.

Basal 'Spikes'.

On March 16, conidia were obtained from the leaves of naturally produced 'spiked' growths occurring on young plants in pots in an unheated glasshouse. They were collected by completely immersing the leaves in distilled water in a small beaker, and when a sufficient quantity had been obtained—as was judged by the black deposit at the bottom of the beaker—the leaves were all removed. After $2\frac{1}{2}$ hours the first zoospores were released, and after $3\frac{1}{4}$ hours very large numbers were in active movement in the water.

Twenty-one young hop plants of Hallertau,[1] Aischgrund,[1] and Tutsham, varieties known to be susceptible to Downy Mildew, were used for inoculation; these were being grown in another unheated glasshouse, and were brought into the laboratory for preliminary preparation and for observation of the condition of the buds. The top earth in the pots was loosened and removed in order to expose the upper portion of the hop sets and buds. These buds were in various stages of development; some were dormant, but several in each pot were elongating, and in a few there were shoots up to 17·8 cm. high.

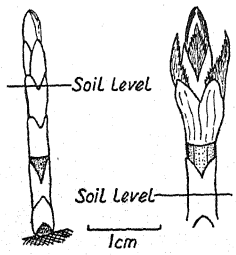
Owing to the fact that not all the buds on a hop set necessarily grow out in any one season, choice was made in the case of each plant, of one bud only (or young shoot) which had already slightly elongated. The smallest of these buds was 2·0 cm. and the largest 4·0 cm. in length. Dried *Sphagnum* moss, made wet with tap water, was thoroughly squeezed by hand pressure to remove excess moisture, and a small quantity was wrapped around each bud to be inoculated. The wrapping of moss was held in place by an outer covering of tin-foil, which was pressed in at the extreme base of the bud in order to secure the retention of as much of the inoculum liquid as possible. The cup-shaped tin-foil covering was left open at the top. The moss wrapping extended from near the base of each bud (shoot), throughout its length, and just enveloped the apex.

Approximately 1·0 c.c. of the inoculum, containing numerous zoospores in movement, was dropped slowly on to the moss until the saturation-point appeared nearly to have been reached.[2] Inoculation of all the 21 buds

[1] The varieties Hallertau and Aischgrund had been sent in 1927 by Dr. Hampp, Director of the Hop Research Station, Hüll, Bavaria.

[2] There is a possibility that in a few of the twenty-one inoculations a small quantity of the liquid may have escaped below the tinfoil into the earth of the pot.

(shoots) was completed 3 hours after the first appearance of zoospores in the inoculum. The loose soil, previously removed from the pots, was next replaced, without covering up the moss wrapping, and a small beaker was inverted over each inoculated bud in order to maintain a moist atmosphere. The room temperature during the evening and night following inoculation was 11° – 13° C. Notes were made of the number and height of all additional shoots which were then above soil level in order to obtain a comparison of the rate of growth; these are recorded in Table I.



TEXT-FIG. 1. The one bud on Hop plant C 2 (left) and the one shoot on L 6 (right) as they appeared when the moss covering was removed forty-eight hours after inoculation. C 2 was in the most retarded and L 6 in the most advanced state of development of all the twenty-one buds when inoculated.

As noted above, the inoculated shoots were of different heights; they were also in different stages of development. When the wrapping process was about to be performed each shoot was closely examined with a lens, and if no portion of the lamina of the minute leaves could be observed projecting from the covering stipules, the latter were regarded as 'closed', and the plant was labelled with a prefix 'C' to its number; 12 of the 21 shoots (C 1–C 12) inoculated showed no exposed lamina. Seven shoots revealed a portion of one or more laminae just protruding from the stipules; these (L 1–L 6 and L 8) were regarded as 'leafy'. In two cases the lamina had not grown beyond the stipules, but the marginal serrations were just visible; these intermediate examples were numbered CL 7 and CL 9.

On March 18, exactly 48 hours after inoculation, all the moss and tinfoil coverings were removed and drawings made of the state of development of all the 21 shoots. Text-fig. 1 shows an example of the most backward of these (C 2) and of the most advanced (L 6). Inverted beakers were again placed over the inoculated shoots for a further 24 hours, and were removed on March 19, when all the plants were taken from the laboratory to an isolated and unheated glasshouse otherwise unoccupied. On the 5th day after inoculation (March 21) all the shoots were examined, but no effects were visible. On the 10th day (March 26) a silvery coloration of two small leaves on CL 7 was observed, dense masses of conidiophores and spores were found at the apex of one leaf on L 1, and a yellowish discoloration of the leaves of L 5 and L 6 was noticed.

During the following weeks many inoculated shoots became affected, and when their growth had ceased and withering had begun, the plants were removed from the pots and the underground parts were examined by hand-sectioning to determine whether any mycelial invasion of the set had taken place. Owing to the fact that this stoppage of growth or withering of the 'spike' occurred at different times the final records were not simultaneous,

TABLE I.

The Length of the One Inoculated Shoot and of Other Shoots on each Hop Plant and the Result of the Inoculation.

	One Shoot Inoculated.			Result.			Other Shoots.	
Number and Variety of Hop Plant.	16. 3. 28 Height above Soil (cm.)	Total length. (cm.).	Total height reached above soil (cm.).	On the Shoot.	On the Root-stock.	No.[1]	16. 3. 28 Height above Soil (cm.).	Maximum Height reached. (cm.).
C 1 Aischgrund	0·0	2·8	—	Nil	Nil	2	0·5 1·0	—
C 2 Hallertau	1·0	3·0	2·0	Dwarf Spike	Infected	2	1·0 15·3	64·3
C 3 Hallertau	1·7	3·0	—	Nil	Nil	3	2·5 to 5·0	—
C 4 Hallertau	—	2·0	—	Nil	Nil	3	1·25 to 2·5	—
C 5 Aischgrund	—	2·0	—	Nil	Nil	3	0·75 to 2·5	—
C 6 Aischgrund	2·0	4·0	11·0	Spike	Infected	4	1·25 to 2·0	129·0
C 7 Hallertau	0·7	2·2	2·0	Dwarf Spike	Infected	2	3·2 15·3	69·0
C 8 Aischgrund	2·3	3·7	9·8	Spike	Infected	1	3·8	49·5
C 9 Tutsham	0·0	3·0	4·0	Spike	Nil	9	1·2 to 3·8	92·0
C 10 Tutsham	0·3	2·2	—	Nil	Nil	3	3·8 to 7·6	—
C 11 Tutsham	1·0	3·6	22·8	Late Spike	Nil	4	2·6 to 10·1	151·2
C 12 Tutsham	1·0	2·3	—	Nil	Nil	3	1·3 to 7·6	—
L 1 Aischgrund	3·0	—	13·5	Spike	Nil	2	1·3 2·6	103·7
L 2 Aischgrund	3·0	4·0	81·0	Leaf Infection	Nil	0	Nil	9·8
L 3 Hallertau	2·5	—	2·3	Dwarf Spike	Nil	2	1·3 20·3	72·5
L 4 Hallertau	2·5	—	4·7	Spike	Infected	2	2·6 7·6	76·2
L 5 Hallertau	2·0	—	10·7	Spike	Infected	1	5·1	51·4
L 6 Tutsham	2·5	4·0	11·0	Spike	Nil	3	All 1·9	43·0
CL 7 Tutsham	0·5	2·5	90·0	Leaf Infection	Nil	4	1·3 to 2·5	63·0
L 8 Tutsham	2·5	4·0	120·0	Leaf Infection	Nil	6	2·6 to 10·2	142·0
CL 9 Tutsham	0·8	2·8	14·5	Late Spike	Nil	2	1·3 1·3	201·0

[1] Additional shoots appeared after March 16, and on some plants a few shoots (not inoculated) were removed as growth progressed. Thus on L 1 and C 6 shown in Plate XVII, only one healthy stem was left.

and in some cases it was not convenient to examine the underground parts until the first week of December 1928.

Of the 21 shoots inoculated, 6 remained healthy (C 1, C 3, C 4, C 5, C 10, C 12) and these, with the other stems in the same pots, made normal growth and were entirely free from disease both in their above- and below-ground parts. The other 15 shoots became infected, but showed a variety of symptoms which can be conveniently grouped for descriptive purposes under the four following headings.

1. *Leaf Infection only.*

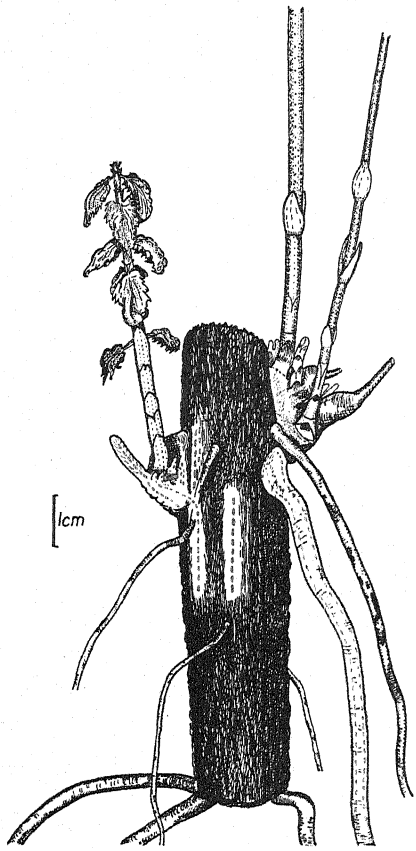
Three of the shoots (L 2, CL 7, L 8) grew normally. During the fortnight following inoculation they had grown respectively to heights of 11·5 cm., 9·0 cm., and 11·0 cm., and it was then observed that in each case the apex of one of the lowermost pair of leaves was of a pale colour, and was densely covered below with conidiophores. On L 2 and L 8 an exactly similar area of infection occurred also at the apex of one leaf of the pair next above. The third (uppermost) pair of leaves on each shoot had recently expanded and was unaffected. Growth of all the stems continued normally, and on April 28 they had reached heights of 81 cm., 90 cm., and 120 cm.; on each of them nine pairs of leaves had expanded.

The areas of infection enlarged somewhat, and finally the lamina in that part turned brown and withered. Only on L 2 did the areas enlarge to such an extent that nearly the whole lamina was killed. The fungus, however, in no case succeeded in reaching the petiole, and since apparently there was neither penetration nor invasion of the main stem, growth was not checked, and the size of the foliage was normal.

Examination of the parts below the soil on December 3 showed that there was no mycelium either in the thickened bases of these stems or in any part of the sets.

2. *Dwarf 'Spikes'.*

In three instances the inoculated shoots (C 2, C 7, L 3) were unable to increase more than about 1 cm., and failed to produce any fully expanded leaves. They were examined on the 17th day (April 3) after inoculation, and though a somewhat sickly appearance was noticeable, the fungus was not externally evident. On the 27th day (April 13) when they were next examined, all three shoots were decidedly pale in colour; three pairs of very minute leaves were only just projecting from the stipules which had enclosed them, and on each shoot from one to four pairs of stipules showed conidiophores on both their surfaces, but more plentifully on the inner. The dorsal side of the ill-developed lamina, where nearly in contact with the infected stipule, also commonly bore conidiophores, and it was evident that if the original



TEXT-FIG. 2. Hop plant L 4 (variety Hallertau) on June 3. Semi-diagrammatic representation showing the 'spiked' shoot (left) resulting from inoculation of a bud with zoospores of *P Humuli* on March 16. The mycelium (red) was present through the entire length of the 'spike', in the one-year-old cushion of tissue from which it arose, and in several buds thereon. In the original set it was present in cortex, phloem, medullary rays, and pith (centre). Hyphae were traced through a distance of 7·5 cm. in one large root (right).

infection had been confined either to stipule or to leaf, it would have been easy for the fungus subsequently to proceed from one to the other.

No further growth took place. On the 37th day (April 23) all the stipules were brown, and the partly grown leaves were no further advanced than ten days previously. On the 42nd day (April 28) all the stipules and the leaves were brown and withered; only the stem showed a trace of green colour. It is worthy of note that the fungus did not occur on any part of the other stems in the same pots.[1]

On May 2, 46 days after inoculation, all the three plants were removed from the pots for internal examination of the root-stocks. In C 2 the mycelium was traced through the entire length of the inoculated shoot (3·5 cm.), and for a distance of 1·5 cm. from its base into the cortex and phloem of the one-year-old part of the root-stock. It invaded this only in a downward direction, and was not present in the one-year-old tissues above the point of origin of the inoculated shoot. The hyphae were also traced for a distance of 1·3 cm. down a root which originated close to the base of the inoculated shoot. In L 3 the mycelium was found throughout the length of the inoculated shoot (3·6 cm.); at the base the pith was hollow, and quantities of oogonia and antheridia were present. The mycelium did not pass beyond the base of the shoot into the tissues of the set. On plant C 7 the hyphae extended the whole length of the shoot (3·5 cm.), and were present in all the parenchymatous tissues of the one-year-old part of the root-stock. Hyphae were present in this to a distance of 1·0 cm. above the base of the inoculated shoot and to 2·0 cm. below. They extended to the base of another stem which had reached a height of 69·0 cm. and which showed no ill effects. Two roots arising from this part of the set were also invaded, one to a distance of 3·25 cm. and the other to 2·5 cm. The length of tissues permeated by mycelium, i.e. from the tip of the shoot to its lowest position in the root, was 8·75 cm. There were two young buds on the diseased part of the set below ground, and although hyphae were close to them, neither was actually penetrated.

3. *Typical 'Spikes'.*

In the following seven cases (C 6, C 8, C 9, L 1, L 4, L 5, L 6) the inoculated shoots became infected and developed symptoms characteristic of 'basal spikes' such as commonly occur under natural conditions in hop gardens. Four of them are illustrated in Pl. XVII. Some of the details of the progress of their diseased condition, e. g. the date of first discoloration due to the fungus, the number of leaves which bore conidiophores,[2] and the

[1] Care was taken when watering to avoid applying any liquid close to the infected shoots.

[2] Many of the leaves produced black masses of conidiophores in spite of the fact that the air in the glasshouse was kept dry in order to hinder any spread of the disease to uninoculated stems. A large proportion of those leaves recorded as not producing conidiophores might have been induced to have done so had the humidity been increased.

height reached by the 'spiked' shoot, are included in Table II. In nearly every case, as growth proceeded, interesting phenomena were observed which throw light on some of the unexplained features of 'spike'-formation in nature.

While it may be assumed that in those plants already described under '*Leaf infection only*' the fungus succeeded in directly infecting the leaves alone, and failed to grow from them by way of the petioles into the main stem, in the present instances it is most probable that general systemic invasion was brought about as a result of primary infection of either (1) the few exposed laminae, (2) the stipules, or (3) any short length of internode. The inoculated shoots on three of the plants (C 6, C 8, C 9) had no part of a lamina visible when the inoculum was applied,[1] and in these cases it is most likely that the stipules were the first to be penetrated.[2]

The presence of the fungus was first revealed either by a pale yellow-green or silvery discoloration of the lamina, or by conidiophores, on one or more leaves of the lowest two pairs (see Table II). In L 6 the discoloration observed on March 26 (ten days after inoculation) involved the laminae of the three pairs then expanded—a fact worthy of notice, since this shoot was recorded on March 18 as being the most advanced, having the third pair of leaves just visible when the *Sphagnum* moss was removed. In L 4, L 5, L 6, the yellow colour first occurred near the apex of the leaves, and then gradually spread towards the petiole, which presumably it entered in order to reach the main stem.[3] L 1 was exceptional in that the fungus was forming conidiophores on an area (again near the apex) without any yellowish discoloration. It spread towards the petiole, however, in a similar manner, and its course was marked by a track of withered tissue. If, as regards C 6, C 8, C 9, the primary infection by zoospores was effected through organs other than the leaves it might be expected that the yellow colour, marking the spread of the fungus in the lamina, would first be observed at the base of the lamina. Such was actually the case in the one leaf of C 6 and in one of the three discoloured leaves of C 9. In the two leaves of C 8 and in the remaining two leaves of C 9, the point of origin of the yellow colour was not determined as its spread had already taken place over the whole lamina when the first notes were made.

The shoots had from four to six leaves, and at the time when the first symptoms of infection were observed they were not completely 'spike'-

[1] Two days later, when the moss was removed, the extreme apices of one pair of minute leaves could be distinguished projecting from the covering stipules.

[2] It has frequently been noticed that the stipules provide a suitable tissue for the growth of *P. Humuli*. The stipules of infected shoots often bear conidiophores before the fungus appears on the laminae of the leaves which they subtend.

[3] The main stem may also have been invaded independently of this, either directly or through the stipules. Invasion of the petiole from the lamina has already been recorded in *P. Humuli* (13, pp. 359, 360) and in *Peronospora de Baryi* (14, p. 55).

like; the main stem was not thickened; there were, in most cases, healthy leaves, and the apex of the shoot appeared unaffected and capable of growth. Within the period of approximately one month (see Table II) there was

TABLE II.

The Progress of the Disease and Rate of Growth of the Seven Stems which became Typical 'Spikes'.

Number and Variety of Hop Plant.	Date.	Number of Leaves.			Height of Shoot above Soil (cm.).
		First Discoloured.	On whole Shoot.	Showing Conidiophores.	
C 6 Aischgrund	Mar. 29	1	4	Nil	3·9
	Apr. 23		10	7	10·5
	28		12	10	11·0
C 8 Aischgrund	Mar. 29	2	4	2	5·1
	Apr. 23		10	10	9·5
	28		14	14	9·8
C 9 Tutsham	Apr. 3	3	4	Nil	3·0
	23		10	6	4·0
	28		10	8	4·0
L 1 Aischgrund	Mar. 26	Nil	4	1	—
	28		4	3	7·0
	Apr. 14		12	12	11·7
	28		14	12	13·5
L 4 Hallertau	Apr. 3	1	6	1	4·7
	23		8	5	4·7
L 5 Hallertau	Mar. 26	1	6	Nil	—
	28		6	1	5·5
	Apr. 14		10	7	9·8
	23		14	9	10·75
L 6 Tutsham	Mar. 26	6	6	Nil	—
	29		8	4	7·3
	Apr. 3		8	6	—
	14		16	13	10·7
	23		18	15	10·7
	28		20	18	11·0

little increase in length, and the number of leaves on any shoot which had been able to unfold was between 8 and 20. The fungus evidently invaded the main stem in both the 'C' and 'L' classes of shoot, and penetrated the laminae of successive leaves soon after these had unfolded. Thus a pair of leaves noted to be healthy and situated next above a diseased pair, were found invaded from the petiole and completely covered with conidiophores on the ventral surface after 25 days. In this time three more pairs of leaves had expanded, and all were exhibiting the yellow mark showing that the fungus had started from the petiole. Of these, all except the uppermost pair were covered with conidiophores on the ventral surface of the lamina. The fungus in the main stem was eventually able to reach the terminal bud,

with the result that growth ceased, and any leaves which were able to expand were already discoloured and often bearing conidiophores when they assumed the horizontal position.[1] The terminal bud itself, including stipules and minute leaves crowded together, was turned a greyish yellow colour, and was evidently so permeated by mycelium that it was incapable of further development. In some cases conidiophores occurred plentifully on the stipules, on the main stem, and on some of the petioles. There was no infection of the foliage of uninoculated stems. After April 28, when it was found that systemic infection was preventing any further growth, and when the lower and longest-infected leaves were brown and shrivelled, no further notes were made until the underground parts of the inoculated plants were examined.

In the one-year-old portions of the sets of L 1 and of C 9 so much damage to the cortex and phloem had been caused by eelworms (*Tylenchus* sp.) that it was impossible to trace with any degree of certainty the hyphae of *P. Humuli*. They were examined on May 21 and December 3 respectively. No mycelium could be found in any part of the set of L 6 (on December 1), and it is certain that no invasion by the fungus had occurred.

In the remaining four cases hyphae had grown down presumably from the inoculated shoot ('spike') into the whole of the one-year-old portion of the root-stock. L 4 (see Text-fig. 2) provided an example of almost complete underground infection; hyphae and haustoria were found (on June 3) throughout the length of the 'spike', in the cushion of tissue from which it arose, and in several buds which were situated on this. Some of the buds were dormant, but one which was 1·0 cm. in length, was permeated as far as the apex, and in it the hyphae were invading every stipule and immature leaf. From this cushion of tissue the mycelium passed into the original set of 1926 growth, now two years old, and besides travelling vertically upwards and downwards it traversed it by means of the medullary rays, and reached the pith which had not rotted, and in which it was traced longitudinally through a distance of 2·0 cm. On the side of the set opposite to the base of the 'spike' a large root was infected by hyphae as far as 7·5 cm. from its attachment to the set. Four other large roots up to 32 cm. long were not invaded. Two healthy stems and several buds close to the infected root were not affected, although the one-year-old outgrowth from which they arose contained hyphae.

The root-stock of L 5 was composed of a slender one-year-old outgrowth from near the base of the original set of 1926. The outgrowth was 6·5 cm. long and only 7 mm. in diameter; it bore at the apex the hardened base of the stem of 1927 growth from which it was itself derived. At the apex

[1] Instances occurred, as they do under natural conditions, in which one leaf of a pair situated between diseased pairs remained free from invasion by the fungus during the whole period.

there were also the 'spiked' shoot and a healthy bine. Mycelium was present in the stem of the 'spike' and throughout the whole length of the one-year-old portion described. In this it was confined to the cortex and phloem, and had not reached the pith; it was present at the extreme base of the healthy bine, but had not invaded it. The whole of the older (1926) set was free from infection, including two large buds which it bore near the soil level.

The root-stock and extent of infection in C 8 were very similar to the last. Here again a one-year-old outgrowth from the base of the original set was permeated by mycelium from the 'spike' at its apex, to the point of junction with the older set—a distance of 7·0 cm. This outgrowth was stouter than in L 5, and measured 14 mm. in diameter; hyphae were present in the cortex, phloem, and pith. In addition to the 'spiked' shoot, a healthy bine (shown in the photograph, Pl. XVII) was growing from this part of the root-stock, and it was remarkable that whilst showing no outward signs of attack, this stem was penetrated by hyphae from its base upwards, in pith and cortex, to a height of 7·5 cm.[1] Numerous oogonia and mature oospores were found in the cortex. While the remote possibility of external infection of this apparently healthy stem by zoospores exists, the writer is of the opinion that the infection resulted from internal invasion by mycelium from the set. This examination was made on May 18, exactly nine weeks after inoculation of the neighbouring shoot had been performed.

Only a small amount of one-year-old growth occurred on the set of C 6; this, together with the whole length of the 'spiked' shoot, was completely invaded by mycelium, which also penetrated the original set (of 1926 growth), not only through a distance of 2·0 cm. in a downward direction, but also transversely in the medullary rays. The infection of this older part, however, had not progressed sufficiently far on June 3 when the examination was made, for another one-year-old outgrowth on the other side of the set to become invaded; the buds and one long stem (129 cm.) originating from this were consequently free from hyphae.

4. *Late or Delayed 'Spikes'.*

Two of the inoculated shoots (C 11 and CL 9) developed into 'spikes' of a character differing somewhat from that of the shoots already described. The symptoms of infection, their tardy appearance, and consequently the greater amount of growth of the stem before becoming truly 'spiked' were the main points of difference. Such diseased shoots as these are not exceptional under natural conditions; their conspicuousness has for some years induced speculation as to the causes leading to their formation.

On April 3 the inoculated shoot of C 11 had been examined, and owing

[1] The total height of the apparently healthy stem was 49·5 cm., and eleven pairs of leaves were expanded.

to its normal appearance, there was no suspicion that it was infected. On April 13, when the shoot was 22 cm. high with four pairs of large leaves, it was observed that one of the lowest pair and both of the next two pairs (i. e. 5 leaves) were marked with a narrow and irregular band of yellow colour on the midrib itself and on each side of it. This colour was visible on both surfaces, and in every case started from the base of the lamina, obviously indicating that the fungus was beginning to invade by way of the petioles. Below each yellow area, conidiophores were present and appeared black or grey according to the quantity. The discoloration did not extend far from the petiole, and in no case reached farther than half way towards the apex of the lamina. The petioles of the five affected leaves were thickened, as were also those of the next pair above (fourth pair). The laminae in this latter case, however, though they were small, showed no external signs of disease. On April 23 no change had occurred in the yellow areas already noted. One of the fourth pair of leaves was still healthy, but at the extreme base of the other, a minute yellow area was beginning to appear at the place of junction with the petiole. A fifth pair of leaves had by now become horizontal; one was apparently healthy, the other showed a small coloured area close to the petiole and situated on each side of the midrib. The main stem between the third and fourth pairs of leaves was blistered, and in a damper atmosphere would probably have produced conidiophores. The growth during the 10-day interval had been only 5 mm. On April 28 the five discoloured areas first recorded had not increased in size, but had become entirely brown. The fungus had apparently been unable to push its attack farther into the laminae; on the main stem the raised whitish blisters were more conspicuous, but no conidiophores were produced.

TABLE III.

The Progress of the Disease and Rate of Growth of the Two Stems which became 'Delayed Spikes'.

Number and Variety of Hop Plant.	Date.	Number of Leaves.			Height of Shoot above Soil (cm.).
		Discoloured.	On whole Shoot.	Showing Conidiophores.	
C 11 Tutsham	Apr. 13	5	8	5	22·0
	23	7	10	5	22·5
	28	9	14	5	22·8
CL 9 Tutsham	20	6	14	0[1]	14·25
	28	8	16	4	14·5

[1] Conidiophores were present on the stipules below one pair of leaves.

During the winter (on December 4) when the root-stock was examined, mycelium and the characteristic haustoria were found throughout the length of the pith [1] in the affected shoot, but no hyphae had entered any living part of the root-stock.

The production of this particular form of 'spike' under controlled conditions is of special interest when the state of the bud on March 16 is considered. At that date there was no sign of extrusion of any lamina; further when the *Sphagnum* moss was removed on March 18 there was still no vestige of any leaf and the stipules were tightly closed.[2] From the above evidence it may be deduced that the zoospores first penetrated either (1) the stipules, or (2) the minute internodes then formed.[3] The mycelium would gain an entry to the main stem and, according to its rate of growth in relation to that of the shoot and its position in the tissues of the main stem, would prevent further development at the growing-point and would penetrate the petioles of leaves, one by one, and eventually reach the laminae. The facts observed in this case (C 11) and in the previous instances of a similar nature (C 6, C 8, C 9; p. 690) lend support to this supposition since the first appearance of the fungus was at the basal end of the laminae.

The last form of 'spiked' growth to be considered, on CL 9, is an example of long-delayed action by the mycelium of the fungus. Up to April 20 the growth had been normal and four pairs of perfectly healthy, large leaves had been produced; on that date, however, when the stem had reached a height of 14·25 cm., the next three pairs of leaves were found to be small, down-curled, and yellow-green over the whole lamina, except at the margins, while the terminal bud was also pale yellow in colour.[4] All the plants had been examined a week previously (April 13) and CL 9 had shown no symptoms of disease; it is certain therefore that the three uppermost pairs of leaves became invaded, presumably from within, and showed the symptoms in a period of 8 days. Conidiophores had not appeared on the laminae, but were present plentifully on the stipules below one pair of the discoloured leaves. Eight days later a further pair of leaves had separated from the apical bud, and these were similarly discoloured. Conidiophores had by then formed a dense black covering on the lower surfaces of the laminae of the lowest two pairs of discoloured leaves (which had become silvery yellow) but were not present elsewhere.

[1] Parts of the hop plant which have become dry and which are suspected of having been infected can be sectioned in water, or preferably alcohol, and the sections placed in aqueous (alkaline) azo blue. Hyphae and haustoria of *P. Humuli* can be readily differentiated.

[2] Cf. footnote 1, p. 690.

[3] Had the zoospores infected the immature leaves within the covering stipules, this infection would have been evident before April 13 on some of the eight leaves which had by then been long expanded.

[4] The contrast in colour between the three upper pairs of leaves and the four lower pairs was so striking that the infected tip could be distinguished several yards away.

The shoot having by this time developed into a 'spike', in that its apical parts were diseased, no further records were made until May 22, when the plant was turned out of the pot for examination of the root-stock. No further growth had taken place, and no more leaves had expanded. The whole of the diseased apical portion of the stem was yellow, with its four pairs of small leaves brown and withered; this was 4·0 cm. long as compared with a length of 12·5 cm. of healthy green stem below it.[1]

The fact that more than a month elapsed from the time of inoculation to the first appearance of disease symptoms is the most interesting aspect of this case. That the symptoms, when they did appear, were due to any later and accidental infection by conidia dispersed in the glasshouse is highly improbable because in this same pot there were two long stems, respectively 201 cm. and 160 cm. high, having 16 and 15 pairs of leaves expanded. These were both healthy during the whole period of the experiment, as indeed was all the foliage on the numerous other uninoculated shoots in the glasshouse. Had there been any promiscuous infection from the conidia (which were certainly plentifully produced in the house) it might be expected that the 'angular-spot' stage of the disease would have become general,[2] or that stems other than those inoculated, would also have become 'spiked'.

When this shoot (CL 9) was inoculated on March 16 there was visible only the extreme apex of the immature lamina of the lowermost pair of leaves. All the rest were firmly enclosed in the apical bud. The shoot was 0·8 cm. high (from soil level), and the two other shoots in the same pot were both 1·3 cm. high, and in the same condition as regards leaf-development as the shorter shoot. The difference in the heights finally attained by May 22 was undoubtedly due to systemic infection by the fungus. Since, as has been observed, the lowest four pairs of leaves never showed any sign of infection, and the petioles were long and normal, it is presumable that penetration was effected (as in the 'C' class) by way of the stipules or the young internodes and that the hyphae grew with the shoot until, after the fourth pair of leaves had been fully expanded, they were able to invade the petioles and laminae of the next three pairs in addition to the apical bud. When the 'spike' was examined on May 22 mycelium was found throughout its length but not in any part of the 'set' from which it arose. From the base as far as the node at which the fourth and uppermost healthy pair of leaves originated (a length of 12·5 cm.) the hyphae were confined to the pith; above this point as far as the apex, the hyphae were present in all parenchymatous tissue. The facts observed appear to indicate that the mycelium when once in the pith and enclosed by the wood-cylinder, was

[1] The total length of the shoot, from its junction with the root-stock, is considered here. The proportion seen above ground was 4·0 cm. : 10·5 cm.

[2] The atmosphere was never very damp and, in watering, the foliage was never wetted.

unable, during the rapid growth of the shoot, to find a way to the exterior through the medullary rays.

Basal 'Spikes'—Discussion.

The results obtained show that diseased shoots, corresponding to the basal 'spikes' occurring under natural conditions, can be produced by inoculating with active zoospores the young elongating buds or shoots on the crown of a hop-'set'.

Penetration of such rudimentary laminae as are exposed appears to be readily effected by the zoospores, though in some cases where the mycelium does not succeed in growing down the petioles, no systemic infection results. All of the nine shoots showing any trace of lamina at the time of inoculation became infected. Penetration of the stipules or of the young stem itself appears to be an additional or alternative method, though where no lamina is exposed, a greater difficulty is presented and a high proportion of buds resist infection. Of the twelve buds inoculated when without laminae, six remained healthy. It may be pointed out that buds on hop-'sets' grown in pots are not likely to be so elongated when they penetrate the soil as they would be under conditions of commercial cultivation.

The different results of the inoculations have been placed according to outstanding characteristics, in four groups, and hypotheses to explain why these were obtained have already been formulated when considering each case.

The plants were of three varieties, all known to be susceptible, and none of the four groups of results can be associated with any one variety in particular. As will be seen from the analysis presented in Table I, five positive results were on the variety Hallertau, four on Aischgrund, and six on Tutsham. The negative results are evenly distributed between the varieties. The dwarf 'spikes', found only in the variety Hallertau, might appear to be correlated with the known extreme susceptibility of that variety, but the number of instances is small. All three varieties are represented in the group of seven typical 'spikes'.

Invasion of the root-stock was by no means general, but it occurred in six of the twelve plants on which 'spikes' were produced (see Table I). The possibility exists that these particular 'sets' might already have been permeated by mycelium which had hibernated since 1927, but had this been the case it seems reasonable to expect that some shoots, other than the one inoculated, would also have become 'spiked' on each plant.[1] Further evidence against the above supposition is afforded by the healthy condition of the root-stocks of the nine plants on which no 'spikes' were produced (see Table I).

The question as to how far zoospore-infection of the young shoots and

[1] This assumes that the mycelium can grow up from the 'set' into the shoot.

the consequent production of 'spikes' (here obtained by an artificial method) are met with in nature must be considered. It is obvious that the earliest 'spikes' of the season cannot result from infection by zoospores unless the latter are produced in the germination process of oospores, and since nothing is at present known of this process (see, however, the addendum), any other likely cause of infection must be examined.

It is well known that mycelium of *P. Humuli* can exist, in this country at least, in the buds and underground parts of the hop plant and most commonly in the one-year-old parts.[1] Setting aside the possibility of infection by the products of oospore-germination, it is reasonable to suppose that the very first 'spikes' of the season may be caused by mycelium which has hibernated in the bud or in some part of the root-stock. As to the later basal 'spikes', however, which continue to be formed throughout the season, often in great numbers[2] on any one affected plant, some of these may again be due to mycelium which has hibernated, but in the light of the evidence of the present work as to the ease with which they are formed by external infection by zoospores, a more likely explanation is that some of the numerous healthy buds and shoots on the hop root-stock become infected by zoospores during showery weather.[3] These zoospores can be produced in enormous numbers by the masses of conidia borne on the first 'spike' of the season, which, being situated immediately over the root-stock, is in the best possible position for distributing zoospores in the soil. Another source of conidia (which might be carried down by rain to the soil in early summer) is the 'angular-spot' stage of the disease which occurs on the foliage of otherwise unaffected stems.

It was owing to the knowledge that basal 'spikes' may be formed by zoospore-infection of buds that emphasis was recently laid (16) on the necessity for still more prompt removal of the very first 'spiked' shoots of the season.

Terminal 'Spikes'.

During the month of May 1928 similar methods were used in an attempt to produce terminal 'spikes' on vigorously growing healthy bines (stems) in the open.

[1] Mycelial infection of the root-stock has already been recorded (12, 15, 18). Blattny (2, p. 217) states that he has so far failed to discover it in Germany. Zattler (19, p. 19), on the other hand, records it as an occasional occurrence. A preliminary note (4, p. 194) announcing that Millasseau has found mycelium in the underground parts of hops from Burgundy has recently been published. The infection of buds has been recorded (12, 15) in this country; in Germany, Blattny (2, pp. 101, 298) mentions it as a rare occurrrence in the autumn of 1926, and Arker (1, p. 15) records hibernating mycelium in the buds of sets during the winter of 1926–7.

[2] As many as forty have been counted.

[3] Arker (1, p. 16) has been led to much the same conclusions. He does not, however, describe any experiments.

On May 25 one bine, from 78 cm. to 145 cm. long, on each of thirteen plants [1] (ten male and three female) growing in the experimental hop garden at Wye, was selected, and damp *Sphagnum* moss was placed round the extreme tip. The moss covered approximately 5·0 cm. of the stem (measuring from the apex) and the end of the apical bud was left just visible within the covering (see Text-fig. 3). Each wad of moss when applied was wrapped round with tin-foil, and the whole was secured to the hop string (up which the bine was climbing) by means of half-inch rubber-tape.[2]



TEXT-FIG. 3. The tip of a rapidly-growing hop stem (T 129) 86·4 cm. high, showing its state of development when inoculated on May 25. The dotted line represents the lowest position covered by the moss carrying the inoculum. The other twelve inoculated were similar to this.

Conidia were collected in about 70 c.c. of distilled water from the leaves of a basal 'spike' and from some angular spots on larger leaves. By examination of samples drawn into a watch-glass it was found that zoospores escaped after one hour. The room temperature was 15° C. A great many zoospores were active after 2½ hours, and inoculation was then begun in the hop garden. The weather was warm and sunny though not hot. About 2·0 c.c. of inoculum was dropped on to the *Sphagnum* moss until it was thoroughly wetted, but sometimes more if the moss appeared to have dried during the interval of preparation of the inoculum (3½ hours). In one or two cases the liquid trickled for a distance of about 3·0 cm. below the moss, but it was immediately dried off. The inoculation of the thirteen stems was completed in half an hour, and at the finish the zoospores were found to be still active.[3] Test-tubes, previously whitened outside with lime caseinate, were inverted over the tin-foil in order to preserve the moisture.[4] All the tubes and the moss wrappings were taken off on the following evening (May 26) after 27 hours, and it was noticed that the moss was still damp when removed. Previously to this, every inoculated tip was marked by tying a piece of wool around the bine immediately below the

[1] These were seedling plants of various origins.

[2] Thin rubber tape was found to hold securely without slipping. This support was necessary to prevent breakage of the brittle bine under the weight of moss and tinfoil.

[3] The history of the zoospores is as follows: May 25; 2.0 p.m. Conidia in water. 3.0 p.m. First emergence of zoospores. 4.30 p.m. Maximum number active (approximately). 9.0 p.m. Still active; some germ-tubes 60 μ long. 11.0 p.m. Some still moving; numerous germ-tubes 90 μ long. May 26; 9.0 a.m. Some still moving; masses of germ-tubes on surface of water visible with naked eye. 10.0 p.m. All zoospores germinating; old zoospore wall approximately 10 μ diam.; germ-tubes approximately 110 $\mu \times 3$ μ. The room temperature throughout was 15° C.

[4] These were held in position by rubber tape attached to the hop string. The bore of the tubes was 2·0–2·5 cm., and the moss and tin-foil fitted closely into them.

moss wrapping. Text-fig. 3 is a sketch of one of the inoculated tips. The bines were left to grow up the strings without interference, and exposed to the same external conditions as the uninoculated stems on the same plants (see Table IV).

On June 13 (nineteen days after inoculation) all the plants were examined, and notes were made of the condition of bines at every node and of the height reached (see Table IV). The first symptoms of disease were visible on several of those inoculated. The shortened internodes, short thick petioles and smaller crowded leaves typical of 'terminal spikes' were evident on three plants, T 106, T 94, T 59. The apical growth of the remaining ten was not in any way hindered, but in six of these there were symptoms of disease at one or more of the nodes between the wool mark and the tip of the bine. These symptoms consisted of shortened and thickened petioles, short laterals, and in a few cases yellow streaks on the laminae of one or both leaves at the node. The four plants showing no disease on June 13 remained healthy throughout the season. The external symptoms on the infected bines remained for some weeks without alteration, and it was thought advisable to make an internal examination when the diseased parts showed signs of withering. The results are described below.

Terminal 'Spikes'.

The height reached by every bine on the plant was recorded (see Table IV) before the one affected was taken away to the laboratory. T 59 was examined on July 3, T 106 on July 11, and T 94 on July 30. In all three cases typical terminal 'spikes' had been produced; that on T 59 is shown in Pl. XVII.

It is noteworthy that the lowest position of visible symptoms included in the part evidently 'spiked', was in all cases at the third node above the position of the wool. It is therefore probable that infection by zoospores did not take place on the more mature leaves of the length of stem inoculated, but that it occurred somewhat higher, probably in the upper two or three centimetres of the five centimetres inoculated; subsequent internal examination confirmed this assumption (see Table V).

In two cases mycelium extended from the apical bud, down the main stem through nodes and internodes continuously, a distance of 43·2 cm. in the one (T 106), and 45·2 cm. in the other (T 94), and stopped at the second node above the wool (see Text-fig. 4). In the infected part the laterals had never grown out or were very short, shrivelled, and brown; all the petioles were thick and short and invaded by hyphae, but only a few leaves showed the yellow marking indicative of the presence of the fungus. Pale-coloured blisters, due to mycelial eruptions, occurred here and there on the stem. In sharp contrast to the higher nodes described—and just as they do below naturally-occurring terminal 'spikes'—long healthy laterals arose

TABLE IV.

Showing the Hop Plants Inoculated on May 25 for the Production of Terminal 'spikes', and the Number and Height of their Bines.

Ref. No. of Plant.	Height [1] of Bines on May 25 (cm.).	Position of Wool marking Inoculation on June 13 (cm.).	Height of Bines on June 13 (cm.).	Height [2] of Bines on July 30 (cm.).	Result.
T 129	86·4	95·7	230·5	457·2	Inter [3]
	109·2		265·6	457·2	
	106·7		263·1	457·2	
T 120	78·0	88·2	213·3	548·6	Inter
	106·7		195·6	457·2	
T 106	83·8	80·0	137·2	137·2 [4]	Spike
	111·8		217·6	365·8	
T 101	114·3	136·4	274·3	—	Healthy
T 99	118·7	124·4	257·3	365·8	Inter
	83·8		180·3	365·8	
	81·3		213·3	365·8	
	119·4		228·7	365·8	
	93·2		283·7	365·8	
T 97	144·8	150·6	332·8	548·6	Inter
	147·3		311·7	548·6	
	86·3		217·6	[5]	
T 95	127·0	134·7	292·1	457·2	Inter
	162·6		360·0	485·6	
T 94	111·8	131·3	210·2	210·2	Spike
	144·1		325·2	365·8	
	104·9		218·4	365·8	
	83·1		132·1	[5]	
T 86	116·9	127·0	276·8	548·6	Healthy
T 76	106·7	116·8	256·2	457·2	Inter
	159·3		352·4	548·6	
	71·1		177·8	396·2	
	59·2		154·9	365·8	
	56·7		147·4	365·8	
T 59	126·2	134·7	245·3	245·3 [6]	Spike
	104·2		256·2	485·6	
	95·7		225·3	485·6	
	76·2		195·7	406·4	
	63·5		121·9	260·5	
	55·1		124·4	292·1	
T 47	123·7	129·5	269·2	396·2	Healthy
T 24	104·2	113·6	264·9	396·2	Healthy

[1] On every plant the first measurement in this column indicates total height of the inoculated bine. The wool mark was approximately 5·0 cm. below the tip of the bine.

[2] Healthy bines were measured to the nearest foot and 'spiked' bines to the nearest inch. The figures have been converted into centimetres.

[3] The stem did not become infected near the apex, but intermediate zones of infection occurred.

[4] The final measurement of T 106 was made on July 11.

[5] Stem broken by the wind.

[6] The final measurement of T 59 was made on July 3.

from the first and second nodes above the wool[1]; in T 94 one of these laterals measured 2·2 metres, and had reached the top wire in the hop garden. In the third case (T 59) the mycelium was not continuous; it extended downwards from the apical bud for a distance of 40·7 cm., and was followed by a length of healthy internode (10·9 cm.). A further length of stem (14·5 cm.) below this was also permeated, constituting an isolated infection in and around the third node above the wool. The hyphae extended for 10·2 cm. below this node and for 4·3 cm. above it, and invaded the one petiole and the one lateral on one side of the node but left the other petiole and lateral unaffected. The lateral invaded by mycelium measured only 17·8 cm. whereas the other opposite healthy lateral measured 83·8 cm. in length.[2] The invaded petiole was 5·0 cm. and the healthy one 6·5 cm. in length. Above the healthy portion of the internode the fungus had infected the entire shoot with its laterals and petioles.

Oospores occurred in great numbers in the pith of all three 'spiked' stems. Conidiophores did not appear on any of the leaves, perhaps owing to the very dry weather, but they were occasionally present on the stipules. The distances between the wool mark on the stem and the lowest hyphae were as follows: T 59, 45·7 cm.; T 94, 34·3 cm.; T 106, 17·8 cm. The significance of the fact that infection of the stem never occurred so far as the lowermost limit of the inoculation zone, is discussed later.

Intermediate Zones of Infection.

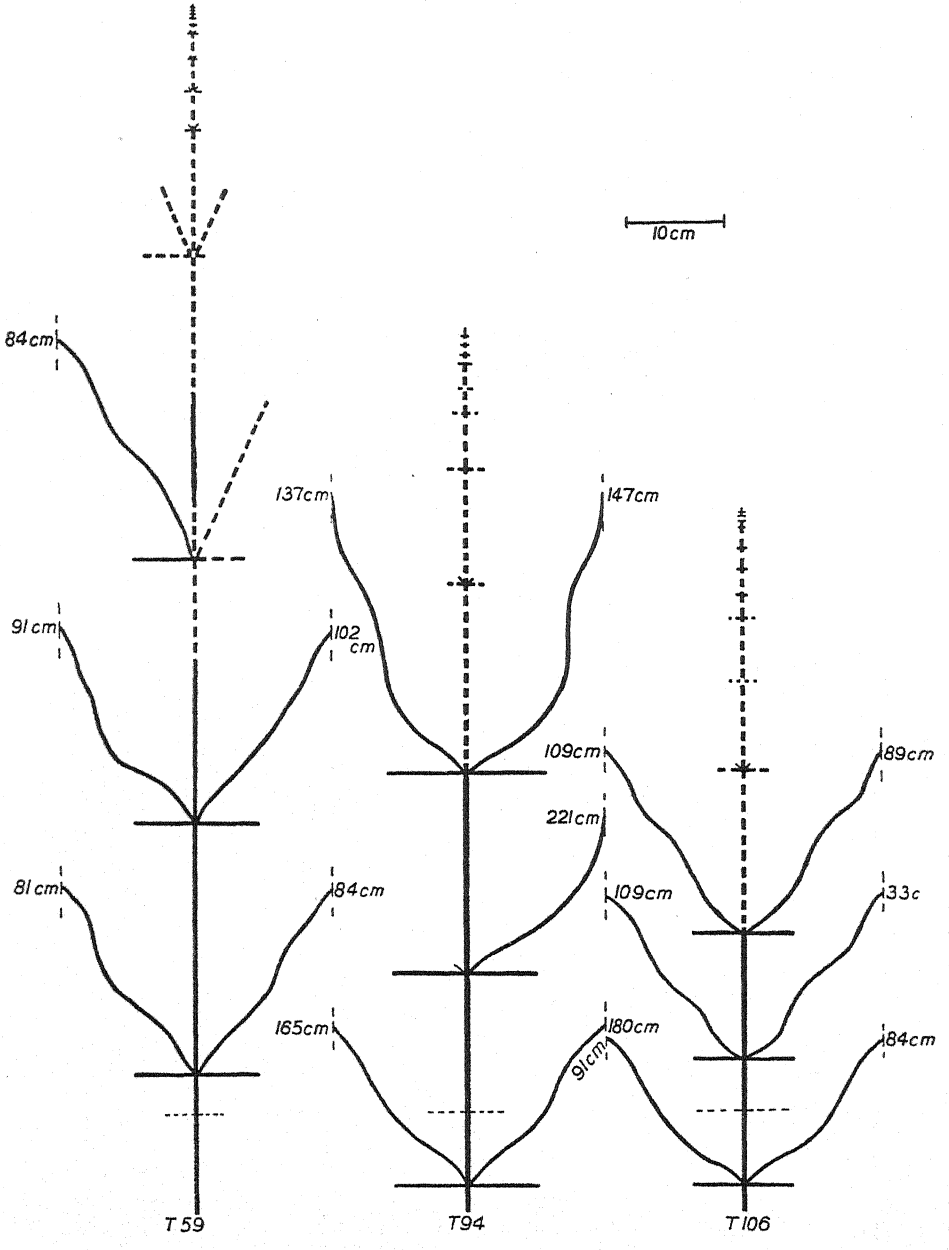
The following six stems (T 129, T 120, T 99, T 97, T 95, T 76) had all reached the top wire in the hop garden (3·6 metres) or had grown beyond it when they were removed for examination on July 30. The tip of none of them showed any sign of the formation of a terminal 'spike', but the symptoms already noted on June 13 were still in evidence.

The results of the internal examination of these stems are shown in Text-figs. 5 and 6, and in Table V. Four of the six will be described in detail.

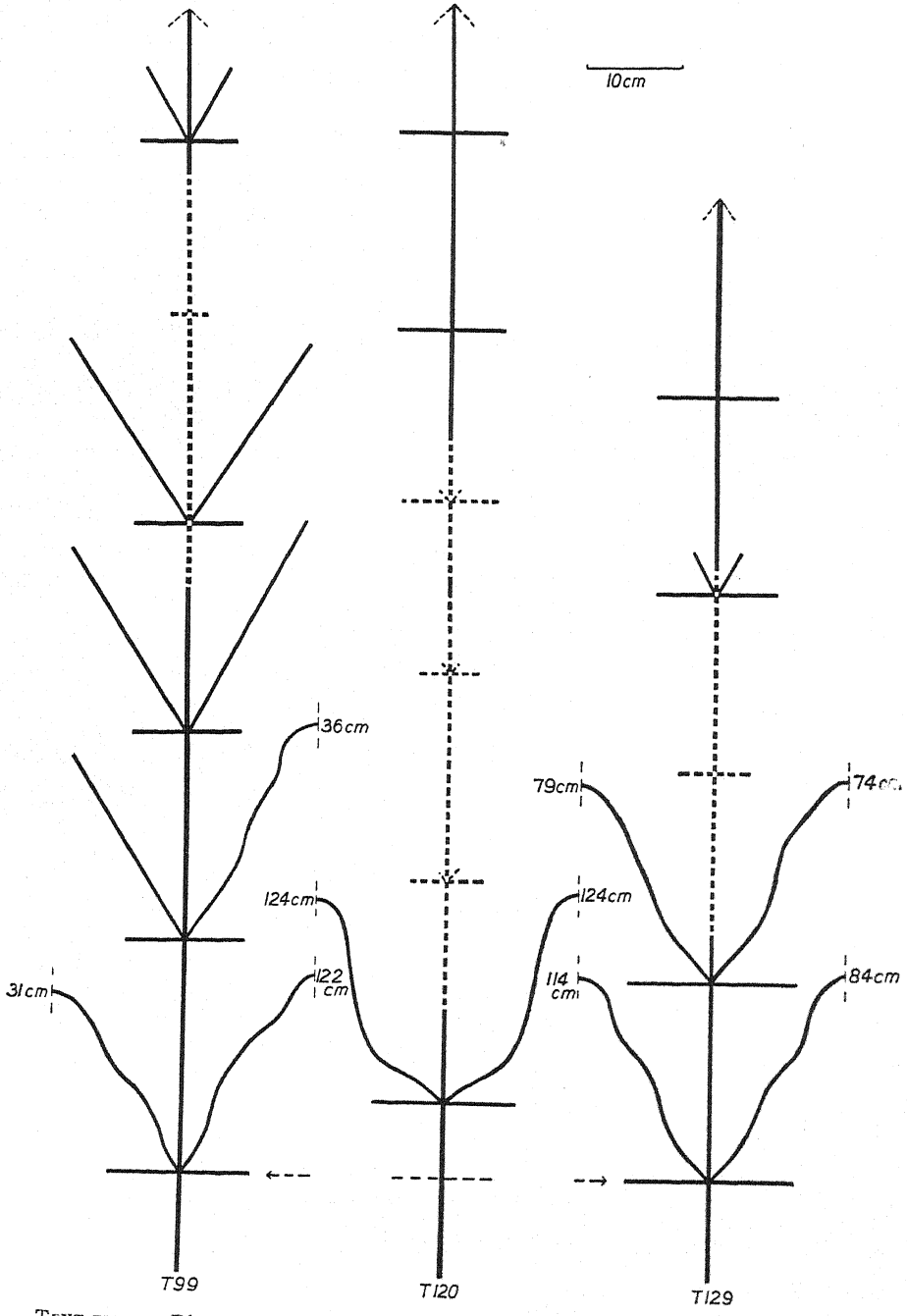
The wool on T 120 was tied on an internode at a place which was 7·5 cm. below a node. Both the internode and node were healthy, and from the latter arose healthy petioles and laterals 1·2 metres long. The internode above this was healthy to a distance of 10·2 cm., but at this point mycelium was found extending to the second node through a length of 12·7 cm. This node was permeated by mycelium, the infected petioles were only half the normal length (3·8 cm. compared with 7·6 cm. at the node below) and the laterals were brown, shrivelled, and dead (1·0 cm. and 2·0 cm.). The next internode, measuring 21·6 cm., was also infected to the node (third above the wool),

[1] These were the nodes situated immediately below the 'spike'.

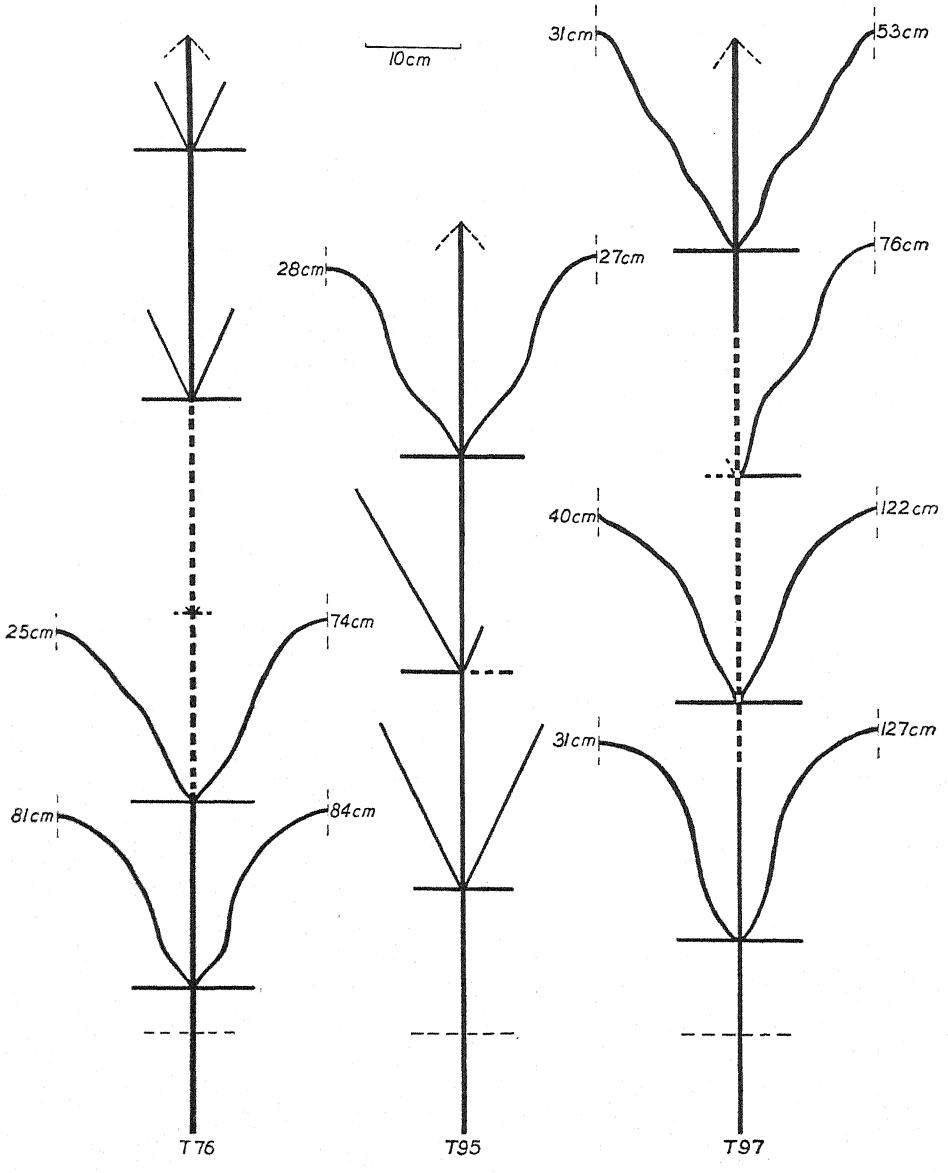
[2] These laterals are shown in the photograph reproduced in Plate XVII. The longer is supported by a bamboo cane.



TEXT-FIG. 4. Diagrammatic representation of the three inoculated hop stems T 59 (left), T 94, and T 106 (right), showing the lowest position occupied by the moss carrying the inoculum (- - - - -), the extent of the resulting systemic invasion by mycelium (heavy dotted lines) and the length of petioles and lateral shoots. Only in these three did the apical portions become terminal 'spikes'.



TEXT-FIG. 5. Diagrammatic representation of the three hop stems T 99 (left), T 120, and T 129 (right) as in text-fig. 4.



TEXT-FIG. 6. Diagrammatic representation of the three hop stems T 76 (left), T 95, and T 97 (right) as in text-fig. 4.

and here the condition of petioles and laterals was similar to that at the node below. Above this, the mycelium continued into the next internode, but ceased after 7·6 cm. when there was a short length of 2·5 cm. of perfectly healthy stem. Hyphae were then again found through the remaining 7·6 cm. of that internode and invading the fourth node above the wool mark. Here

the laterals and petioles were similar to those described. The internode above this measured 17·8 cm., but the hyphae did not extend beyond 6·4 cm. and the rest of the long stem was healthy.

The mark on T 99 was tied at a node which, together with its laterals, measuring respectively 30·5 cm. and 122·0 cm., was free from disease. The internode above (24·2 cm.) was healthy, as was also the first node with its laterals, measuring 23·0 cm. and 35·6 cm. The next internode (21·6 cm.) and the second node were healthy and the laterals were 23·0 cm. and 25·5 cm. long. The internode above this was free from infection to a distance of 15·2 cm., but the remainder (6·4 cm.) contained hyphae, which passed through the third node without being present in the petioles or laterals which were of the same length and appearance as those at the uninfected node below. Thence the mycelium continued upwards throughout the next internode (21·6 cm.) to the fourth node. This was entirely invaded; the petioles were thick and very short (1·9 cm. compared with 5·8 cm. at the node below), and the buds in their axils were brown and dead. The next internode measured 17·8 cm., but the mycelium ceased after traversing a length of 14·6 cm., and thus failed to reach the fifth node. From this point to its apex the stem was healthy.

The inoculation mark on T 97 was on an internode and 9·6 cm. below the first node where there were healthy petioles and laterals (127·0 cm. and 30·5 cm.). The next internode was 24·1 cm. in length, and of this the lower 17·8 cm. were healthy, but hyphae were met with in the upper part (6·3 cm.) and passing through the second node. Here the petioles and laterals (40·0 cm. and 122·0 cm.) were healthy. Throughout the next internode (22·9 cm.) the mycelium was present, and it passed through the third node where the petiole and lateral on one side were unaffected, but on the other side, invaded. The infected lateral measured only 1·9 cm., with its apex brown and incapable of growth, whereas the healthy lateral measured 76·2 cm. Passing through this third node, the mycelium extended 14·6 cm. up the next internode, the total length of which was the same as the last, 22·9 cm. The uninterrupted length of mycelium in the main stem was 43·8 cm., and above this the stem was entirely healthy.

On T 95 only one petiole was infected, and the fungus did not enter the node. This occurred at the second node, 35·6 cm. above the wool mark and is the example of the greatest localization of the mycelium met with. This petiole was 5·1 cm. in length, but the hyphae were not present at the distal (lamina) end, which was healthy for a distance of 1·3 cm. The lateral in the axil was very short (5·0 cm.), but was not infected. On the other side of the node the petiole and lateral were healthy, the lateral measuring 21·6 cm. and the petiole 6·9 cm.

Results closely similar to those of T 120 and T 99 were obtained with T 129 and T 76; the principal features are recorded in Text-figs. 5 and 6.

Terminal 'Spikes'—Discussion.

Two distinct classes of results were obtained in the nine cases where the stems became diseased, although no observable difference existed between the stems at the time of inoculation. The method of inoculation precludes any possibility of knowing which part of the 5·0 cm. length of shoot was first penetrated, and it is therefore uncertain whether the inoculated tips which produced typical terminal 'spikes', and those in which only intermediate internal infection resulted, were infected at precisely the same point.

The formation of typical terminal 'spikes' is shown to be due to systemic infection following penetration by the germ-tubes of zoospores from conidia.[1] In the terminal 'spikes' produced in the present experiments the infection may have started at the apical bud, and may have spread downwards, or it may have started lower (nearer the wool mark) and spread upwards; whichever the method, further growth of the stem was prevented.

TABLE V.

Showing Position of Mycelium in Relation to the Lowermost Limit of the Inoculation Zone.

Ref. No. of Hop Plant.	Above lowest limit[2] of Inoculation.			
	Number of Nodes Healthy.	Length of Stem healthy before Mycelium encountered (cm.).	Nodes Infected.	Greatest Height reached by Hyphae (cm.).
T 76	2	24·8	3rd	64·2
T 95	All[3]	All	2nd[3]	35·6
T 97	1	27·4	2nd, 3rd	71·2
T 99	2	61·0	3rd, 4th	103·6
T 120	1	17·7	2nd, 3rd, and 4th	76·2
T 129	1	25·4	2nd, 3rd	63·0
T 59	2	45·7	3rd–13th	111·8
T 94	2	34·3	3rd–10th	79·5
T 106	2	17·8	3rd–12th	61·0

A second and theoretical explanation of the formation of terminal 'spikes' was put forward by the writer in 1926 (18, p. 106). It was suggested that mycelium from the root-stock might possibly invade a bud which had started growth, and that the hyphae might be carried by the rapidly elongating bine and be left behind in strands at certain nodes or carried up in their entirety. In the present state of knowledge this appears to be unlikely. Although from the experiments described above it is clear that a shoot can make considerable growth after it has become internally

[1] This was suggested in theory in 1925 (7, p. 127).

[2] The mark was on an internode except in T 99 and T 129.

[3] One petiole only, at the second node, was affected.

infected and before it becomes definitely 'spiked', it seems improbable that any stem over 2 or 3 ft. (60 cm.–90 cm.) high, and ending in a terminal 'spike', can have carried mycelium within it from the bud.

The six cases of intermediate stages of internal infection show that penetration occurred some distance below the growing point, and that the mycelium was never able to overtake it or to retard growth. In the one instance of T 95 penetration of the main stem was never effected. Intermediate lengths of healthy tissue between those infected, such as occurred in T 59 and T 120, are commonly met with under natural conditions;[1] their existence may be accounted for if it be assumed that isolated penetrations by zoospores occurred, and that the mycelium formed did not make union with mycelium from other places of penetration. An alternative explanation is that one whole internode might have been infected when young, and that intercalary growth caused the mycelium to be torn apart into separate units.

An interesting and remarkable result of inoculation of the growing tip is that lateral 'spikes' may be formed, and that buds which should give rise to laterals may be killed outright. This is probably brought about by isolated infection at one node or by spread of mycelium into that node from one higher or lower.[2]

The growth of healthy laterals and leaves from an infected node, such as is recorded in T 97, T 99, T 129, and the growth of one healthy lateral and leaf on one side only of an infected node, as in T 59 and T 97, are also met with under natural conditions (18, p. 100).

It is noteworthy that there was no reduction of length of those internodes which were completely invaded by hyphae and that the invasion of one, or two, internodes was apparently no hindrance to the stem continuing its growth to the top wire at 12 ft. (3·66 metres) from the ground.

The exact amount of downward or upward spread that took place during the period of intercalary growth cannot be determined. The downward spread was not great because no mycelium even approached the spot where the wool marked the lowermost limit of the length of stem inoculated (see Table V). The fact that the lower internodes and nodes, with stipules, leaves, and laterals were healthy shows that the original penetration did not take place through those parts which, at the time of inoculation, were the most differentiated. As with the basal shoots inoculated, no evidence was obtained as to which parts of the growing tip were first penetrated, viz. stipules, leaves, nodes, or internodes.

The dry weather must be held responsible for the absence of conidiophores from the leaves of the terminal 'spikes'. This feature was general

[1] One such case has been recorded (18, p. 98).

[2] Under natural conditions the external infection of lateral buds is probably a more frequent cause of lateral 'spike'-formation.

in the later part of the 1928 season in all hop gardens where 'spikes' were found (16), and has been recorded also in some of the earlier examples investigated (18). In no case did infections of any kind occur on the control bines, or on any of the neighbouring plants in the row.

SUMMARY.

1. In the experiments described, 21 young shoots on the root-stocks of 21 pot-plants of the Hop (*Humulus Lupulus*) were inoculated with a suspension of zoospores obtained from the conidia of *Pseudoperonospora Humuli.*

2. During the course of the experiment, 12 of the inoculated shoots became infected systemically, and their growth was checked to a varying degree; 3 showed leaf-infection only, and 6 showed no result of inoculation. The infected shoots were strictly comparable with the basal 'spiked' growths which occur under natural conditions.

3. In 6 of the 12 plants on which basal 'spikes' were produced, the systemic infection spread and invaded the tissues of the root-stock.

4. In a further experiment, the extreme tips of 13 stems 78 cm.–145 cm. long, each on a separate hop plant, were similarly inoculated.

5. Of these stems, 3 became systemically infected, growth being so suppressed that they exactly resembled the naturally-occurring terminal 'spikes'; 6 became infected at certain points on the stem without hindrance to its growth, while 4 showed no result of the inoculation.

ADDENDUM.

Since this paper was written, Dr. K. Arens has shown (*Phytopath. Zeitschr.*; I, 169-93, 1929) that the oospore can be induced, under laboratory conditions, to germinate with the production of zoospores.

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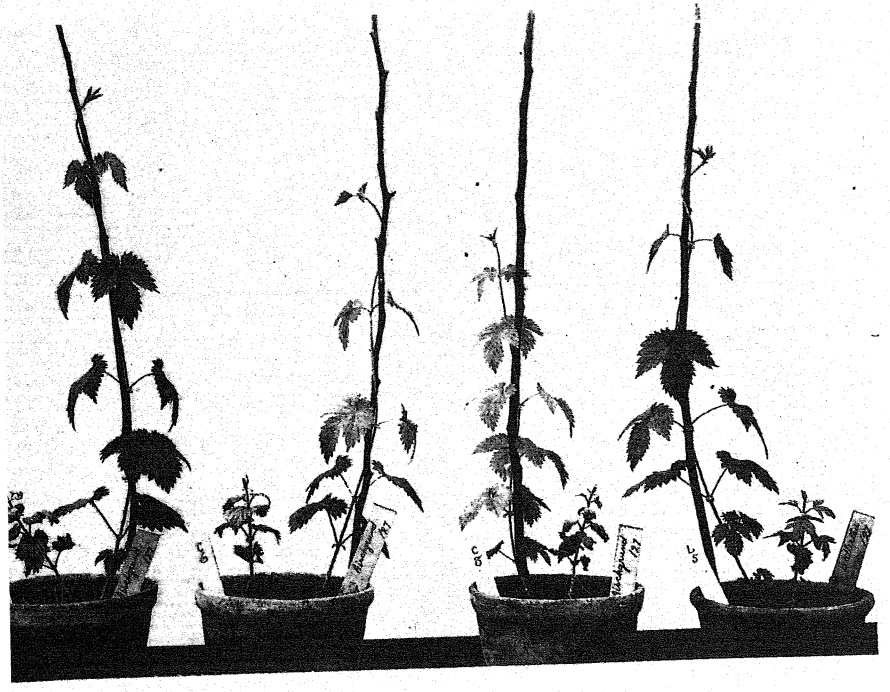
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DESCRIPTION OF PLATE XVII.

Illustrating Mr. W. M. Ware's paper on Experiments on the Production of Diseased Shoots by the Hop Downy Mildew.

Fig. 1. Four hop plants, L 1, C 6, C 8, L 5, on April 17, a month after inoculation of one bud on the root-stock of each, showing the short, basal 'spike' resulting. The dwarfing effect of the fungus (*P. Humuli*) is illustrated by the fact that at this date there were approximately the same number of nodes and pairs of leaves on the 'spike' as on the healthy control stem on each plant. $\frac{1}{6}$ nat. size.

Fig. 2. Terminal 'spike' at the tip of the one inoculated stem on the male hop plant T 59. The tip (similar to that shown in text-fig. 3) was inoculated with zoospores of *P. Humuli* on May 25 when at a height of 126 cm. from the ground. Growth of 119 cm. had taken place when the photograph was taken on July 3. On the left a healthy lateral is seen arising from an infected node which also bears a short lateral 'spike'. The extent of mycelial invasion is shown in Text-fig. 4. $\frac{1}{5}$ nat. size.



1



2

Huth London.



The Epidermal Outgrowths of the Genera Thespesia and Gossypium.

A Morphological Study throwing some Light upon the Evolution of the Hairs constituting Commercial Cotton.

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With Plates XVIII and XIX and twenty-nine Figures in the Text.

THE genera of plants comprised in the Order Malvaceae, if we omit consideration of the Bombacaceae, fall, according to Schumann (1), into four sub-divisions, one of which, the Hibisceae, is characterized by the capsular form of the fruit.

Amongst the Hibisceae, the four genera, *Thespesia*, *Fugosia*, (*Cienfuegosia*), *Gossypium*, and *Thurberia* (*Ingenhousia*), show a closer affinity to one another than they do to other orders of the group. From amongst these four genera, two, *Thespesia* and *Gossypium*, are alone considered in the following investigation.

In order to make clear the position of the various species and varieties here mentioned a scheme of orderly arrangement of the forms is given. It is one that has been found useful in dealing with the numerous types, especially of Old-World Gossypia, that one comes across in field investigations. The scheme does not profess to be a complete system of classification of the cottons. It must be admitted that the assignment of a cotton type to a position in a scheme of classification is often a difficult matter. The recognition even of types themselves can only be made in many cases after very considerable familiarity with them in their natural abode. Away from

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this habitat they often change their characters considerably, and for this reason classification based upon an examination of herbarium specimens must be dubious. The identification of types is greatly complicated too by the readiness with which cottons hybridize. Both the Old-World and New-World forms hybridize freely amongst themselves, but not ordinarily with each other. These two groups must be regarded as entitled to rank as separate species, but whether it be justifiable to make further species, as we have done in our nomenclature, must be admitted to be a doubtful point. Such considerable distinctions as the monopodial and sympodial growth characters have been indicated by Farmer (2), Palladin (3), and others to be but expressions of variation in the water economy of the plant. The forms of *Gossypium* here considered are more especially those of India, and their classification is based on the work of Gammie (16) and Leake (13). The scheme shows the position of all forms mentioned in the text.

(A) Genus *Thespesia.*
Forms with 13 haploid chromosome bodies. — *Thespesia populnea.*
(B) Genus *Gossypium.* — *T. macrophylla.*
(*a*) Old-World forms, with 13 haploid chromosome bodies.
(1) Primitive types. — *G. Stocksii.*
(2) Types with predominant sympodial branching.
I. *G. cernuum* group. An Asiatic group that has become isolated in Assam. Flowers a distinctive pale yellow; bolls large, many seeded; lint hairs short, coarse, and abundant.
II. *G. indicum* group. A common Asiatic group; flowers yellow, lint hairs comparatively long, strong, fine, and scanty. Capsules characteristically incompletely opening when ripe.
III. *G. neglectum* group. A group of hybrid origin between two of the groups I, II, and V. The varieties ring the changes amongst an octave or so of the pairs of the allelomorphic characters of groups I, II, and V. — *G. neglectum* var. *verum*; yellow flower, finer lint, narrow leaf segments.
G. neglectum var. *malvense*, yellow flower, coarse lint, broad leaf segments.
G. neglectum var. *roseum*, white flower, coarse lint, narrow leaf segments.
G. neglectum var. *cutchicum*, white flower, coarse lint, broad leaf segments.
(3) Types with predominant monopodial branching, and tending to be larger plants than (1) and (2) above.
IV. *G. obtusifolium* group. Plants with comparatively few hairs on the vegetative parts. Flowers yellow.

V. *G. arboreum* group. Forms with purple sap extending to the flowers, thus producing red-flowered types.	*G. arboreum.* Corolla with red sap masking a yellow flower. *G. sanguineum.* Corolla with red sap masking a white flower. Of hybrid origin between *G. arboreum* and one of the *neglectum* group.
VI. *G. herbaceum* group. Hairy plants, but not so much so as the intermedium group. Lint hairs comparatively long.	*G. herbaceum* var. *siwa.* From Egypt, a form with its capsules before ripening bright red and not green. *G. herbaceum* var. *goghari* (of hybrid origin).
VII. *G. intermedium* group. Hairy plants, and with dense fuzz on the seed. Lint hairs comparatively scanty.	
(*b*) New-World forms, with 26 haploid chromosome bodies.	
(1) American Upland types.	*G. hirsutum.*
(2) Vine-leafed types. Usually larger than (1), and with more glabrous stems and leaves.	
I. Sea Island cottons. Tall plants with long lint hairs upon the seed coat.	Marie Galante cotton. *G. brasiliense.*
II. Egyptian cottons. Not such tall plants as I, above, and lint hairs shorter. (Perhaps of hybrid origin from a cross of some Sea Island × American Upland.)	Ashmouni cotton.

The larger number of types amongst the Old-World forms, and also perhaps the chromosome numbers, might indicate this group to be more ancient than the New-World forms. The possibility of a polyphyletic origin, however, should not be overlooked. The chromosome numbers have been determined by Denham (4) and Youngman (5).

The two genera *Thespesia* and *Gossypium* are closely related to one another and for this reason it was thought that a parallel study of the outgrowths of the epidermis in the species of the two genera might perhaps throw some light upon certain points in the structure of the cotton hair. *Thespesia populnea*, a small tree-like form, and *Thespesia macrophylla*, an undershrub, were readily available around us. An extensive collection of the *Gossypia*, especially those of the Old World, was equally available in our experimental grounds.

The accounts of previous workers seemed to indicate that a broader study was required than had been made in the past. The economic importance of the mature cotton hair had tended perhaps to focus study too sharply upon its individuality alone, rather than to see if there were not other hairs or forms that threw a light upon its ontogeny and characteristics. In other words, the study had not been a sufficiently comparative one, one was still left with the question—What has been the evolution of the cotton

hair? It was with the idea of trying to answer this question that the present investigation was carried out.

For the sake of convenience the subject will be treated in two parts. Firstly, in Part I, the epidermal outgrowths upon external surfaces of the plant body generally, including the petals, will be described and discussed, and then, in Part II, an account will be given of these structures as found within the fruit capsule.

Part I.

The Epidermal Outgrowths upon External Surfaces.

In *Thespesia* and *Gossypium* there are found upon parts of the plants, excluding for the present all structures inside the fruit capsule, epidermal outgrowths of the following types:

1. Single unbranched hairs.
2. Stellate hairs, and modifications of them.
3. Peltate scales, and their derivatives.
4. Club-shaped bodies.

(1) *The Single Unbranched Hairs on the Plant Body.*

Solitary hairs are found scattered over the plant generally, on the stem, leaves, and floral organs. The single hair of *Gossypium* originates as an outgrowing epidermal cell (Text-fig. 1). These cells do not appear to arise in any pattern or order upon the epidermis, and before the elongation begins nothing can be found to indicate which epidermal cell will take on this outgrowing propensity. In the case of artificially induced hairs on *Tropaeolum peregrinum* Hill (6) states that 'they occupy a position comparable to that of the stomata in relation to the ordinary epidermal cells of the stems'. In the types studied it has not been possible to connect the position of the simple hairs with that of the stomata. At first the cell-wall is thin and contains the protoplasm, nucleus, and vacuoles of an ordinary cell. An examination of the simple hairs upon the petals of *Gossypium* shows that for more than ten days the hair is growing in length, during which time the wall of the cell remains thin. How much longer than ten days is spent in growth in length it is not possible to say, because the time of the actual beginning of the outgrowing of the epidermal cell cannot be decided. After the attainment of full length an increase in the thickness of the wall takes place (Text-fig. 3). This increase in thickness is effected by the daily deposition of a layer of cellulose. Each day a new layer is deposited by the protoplasm on the inside of the cell-wall. Hence we have a primary and a secondary wall. The secondary wall is thus in the form of laminae as described by Balls (9) in the case of the cotton-hair wall. The

old thick-walled hairs upon actively growing organs are deciduous and eventually fall off, new ones arising throughout the growth of the organ.

The thickness of the secondary wall is to be regarded as a property of the hair itself. At a certain age daily deposition of a lamina of cellulose ceases. Exactly what factor in the life of the hair limits this property of increase in thickness it is not possible to say, perhaps it is buried in a past phylogeny. It does not seem to be the cutting off of nutrition due to the thickening of the wall. It may perhaps be connected with a diminution in the volume of the protoplasm, or have been perhaps originally determined by the water economy of the plant. That it is due to the cutting off of nutrition through the increasing thickness of the wall is ruled out, because very definite pits exist in the wall of the embedded portion connecting the protoplasm with that of the neighbouring epidermal cells (Text-figs. 3 and 6). These pits persist as such in the base of the cell and never become obliterated or closed. The wall of the foot or embedded portion thickens like that of the rest of the cell-wall. In this way this cell differs from the surrounding epidermal ones, which only develop thickness in their walls, to any great extent, on that part of the wall exposed to a free surface.

The immediately surrounding ring of epidermal cells whose protoplasmic contents are in continuity with those of the hair-cell often forms a very definite rosette around the base of the hair (Text-fig. 10). Sometimes these cells form a papilla bearing the hair above the level of the surrounding epidermis (Text-figs. 3 and 18). At other times the external walls of the epidermal cells at the base of a hair are level with the general epidermis and then they can be seen to be often marked with very fine line-like striations radiating outwards from the hair as a centre (Text-fig. 4). These striations represent linear depressions in the cuticle (Text-fig. 5).

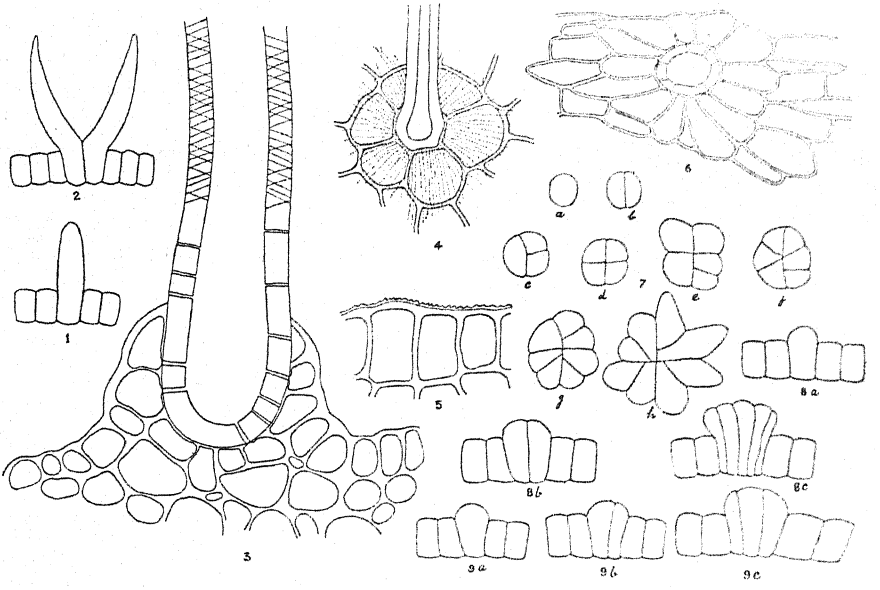
On organs like leaves the single hairs (and also the stellate ones to be presently described) are almost exclusively situated over small vascular bundles or bundle-endings. Where these bundle-endings occur there are no chlorophyll-containing cells beneath the epidermis. The epidermal cells are here elongated in a direction parallel to the leaf surface, whereas the general epidermal cells are more cubical in shape.

All this is very well seen by gently scraping away with a scalpel one epidermis of a leaf and then examining under the microscope the thin portion thus obtained (Text-fig. 10). Lines of elongated epidermal cells, each line consisting of about three cells in width, divide up the whole leaf surface into polygonal areas. These polygonal areas contain chlorophyll corpuscles in their sub-epidermal cells. Beneath the elongated cells there is no chlorophyll.

The close association of the hairs with the bundle-endings is suggestive that their function is, at all events in part, connected in some way with water economy. The following statement by Haberlandt (7) is very

interesting here—'It sometimes happens that the outer epidermal walls are more pervious to water over the veins; this condition may be looked upon as the first step towards the localization of foliar absorption. Almost always, however, it is some specialized form of trichome that is entrusted with the task of absorbing water.'

Along the walls of some cells pits extending through the secondary wall-laminae as far as the primary wall can be seen (Text-fig. 3). At first

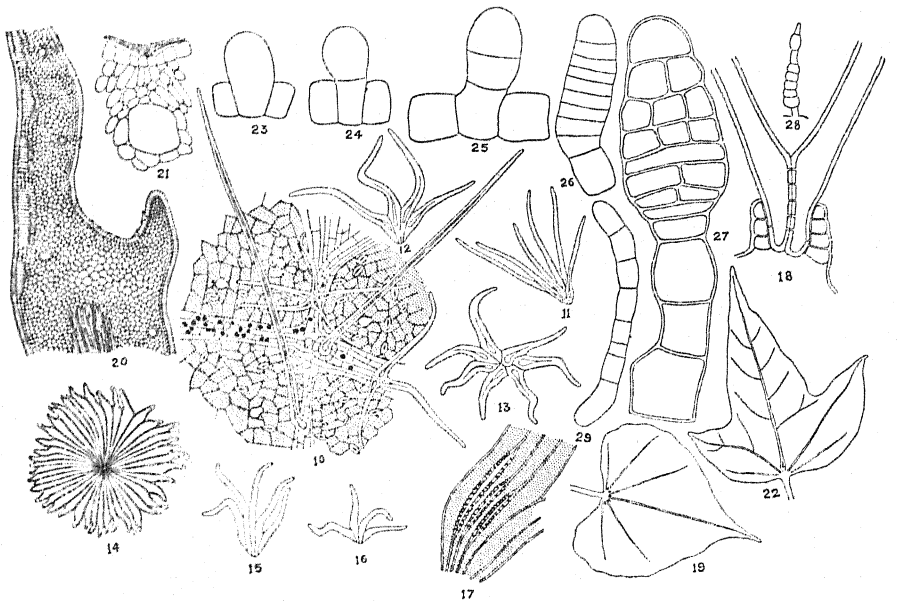


TEXT-FIGS. 1–9. 1. *Gossypium herbaceum.* Outgrowing epidermal cell on the leaf. × 188. 2. *Gossypium herbaceum.* Bifurcate, or two-celled, hair growing out from the leaf × 188. 3. *Gossypium herbaceum* var. *Goghari.* Old hair from leaf petiole showing basal pits extending somewhat above the level of the epidermal papilla, and also shear cracks in the secondary wall. × 188. 4. *Gossypium herbaceum.* Small hair from the leaf, and showing surface striations on the surrounding epidermal cells. × 188. 5. *Thespesia populnea.* Epidermal cells of upper leaf surface, showing surface striations in transverse section. × 188. 6. *Gossypium herbaceum.* Leaf surface over a vein-ending with a big hair cut off transversely at the level of the leaf. Pits in the hair-wall communicating with the tabular cells of the vein-ending are seen. × 94. 7. *Gossypium herbaceum.* *a* to *h* surface views of stages in the division of an epidermal cell of the petal to form a stellate hair-group. × 188. 8. *Gossypium herbaceum.* *a*, *b*, and *c*. Vertical sections of the epidermis of the petal when in the bud, showing cells dividing to form a stellate hair-group. × 188. 9. *Thespesia populnea.* *a*, *b*, and *c*. Vertical sections of the epidermis of the seed, showing cells dividing to form a peltate scale. × 188.

these pits are horizontal but later become oblique. They are true pits just as are those in the base, but differ from them in occurring away from the base, and in having now no neighbouring cell with which to communicate. These pits are more usually seen in the proximal portion of the cell-wall, but they can be found at times toward the tip of the cell. We will speak of these pits, however, as the distal pits, in order to distinguish them from the pits in the base of the cell which facilitate exchange between its contents

and those of the surrounding epidermal cells. In old hairs the distal pits cannot ordinarily be seen.

Some of our types show these pits on the thickened distal wall of the



TEXT-FIGS. 10–29. 10. *Gossypium herbaceum.* A portion of the upper epidermis of the leaf. The tabular cells of a vein-ending, with no chloroplasts in the cells beneath them, are seen intersecting the chloroplast containing areas (shaded). The dark bodies in the cells of the vein-endings are crystals. Club-shaped bodies are seen here and there, and many stomata. × 30. 11. *Gossypium herbaceum.* A stellate hair-group. × 30. 12. *Gossypium Stocksii.* A form of hair-group that occurs upon this plant in addition to typical stellate hairs. Side view. × 30. 13. *Gossypium Stocksii.* As Fig. 12. Surface view. × 30. 14. *Thespesia populnea.* A peltate scale from the petal. Surface view. × 30. 15 *Thespesia populnea.* A derivative of a peltate scale with fewer and somewhat separated rays. From the petal. × 30. 16. *Thespesia populnea.* As Fig. 15. × 30. 17. *Thespesia populnea.* Portion of a shield-shaped derivative of a peltate scale with some of the cells separated in order to show the intercommunicating pits. × 50. 18. *Gossypium herbaceum.* Basal portion of a bifurcate hair from the leaf petiole. Intercommunicating pits are seen in the fused walls. × 94. 19. *Thespesia populnea.* Lower surface of a leaf showing the cavities at the junctions of the main veins. $\frac{1}{8}$ natural size. 20. *Thespesia populnea.* Vertical section of a leaf between two main veins. A cavity is seen in section with the peltate scales upon its wall. × 23. 21. *Thespesia populnea.* Portion of Fig. 20 showing a peltate scale above a mucilage cavity. × 125. 22. *Thespesia macrophylla.* Lower surface of a leaf showing the depression or pit upon the main vein within which club-shaped bodies are found. $\frac{1}{8}$ natural size. 23. *Gossypium cernuum.* Vertical section of the epidermis of the petal when in the bud, showing the first appearance of an outgrowing epidermal cell to form a club-shaped body. × 188. 24. *Gossypium cernuum.* As Fig. 23, but later stage. × 188. 25. *Gossypium cernuum.* As above, old stage. × 188. 26. *Gossypium cernuum.* As above, still older. × 188. 27. *Gossypium neglectum* var. *roseum.* A fully grown club-shaped body, from the petal. × 188. 28. *Gossypium Stocksii.* A modification of a club-shaped body, from inside the capsule. × 30. 29. Ashmouni (Egyptian) Cotton. A modification of a club-shaped body, from the inside of the capsule wall. × 30.

hairs more plainly than others. The pits are, however, difficult of detection upon leaf hairs, and it is not possible to say with certainty that they exist at all on such hairs in some types.

Upon the lower side of the petal of *Thespesia populnea*, near its point of insertion, there occur many comparatively long single-celled hairs. Occasionally coming off from the same place as one of these hairs, and in contact with it at the base, there may be a second smaller elongated cell (Pl. XVIII, Fig. 1). If the walls of these single hair-like cells be carefully examined, especially before they have become fully thickened, some of them will be found to show the presence of pits (Pl. XVIII, Fig. 1). The pits are elongated transversely to the direction of the long axis of the cell, to which the pit is usually obliquely set (Pl. XVIII, Fig. 2). Frequently they occur in groups, especially so in the proximal half of the cell, and the pits in this part are often longer and more easily seen than those more distally situated.

Study of these pits shows that they are places in the secondary cellulose wall of the cell where there has either been no deposition of cellulose, or where, if a deposition has taken place, the cellulose has been absorbed or dissolved away by the protoplasm. The pit, of course, occurs in the secondary wall of the cell and not in the (external) primary wall. Very often a group of pits can be found closely followed by a second somewhat similar group. In a case of this sort if one focuses clearly upon say the first group it will be found that a slight readjustment of the focusing will be required to bring the second group of pits sharply into view. This is exactly what would be required if one group of pits were on the opposite wall of the cell to the other group, that is to say, if one group were on the portion of the tubular cell-wall nearer to the microscope objective, and the other on the portion of the wall further away. Whilst pits do occur at intervals all around the cell-wall the reason for the necessity of readjustment of the focusing, in the case under consideration, is not the suggestion just made, but depends on the fact that the pits in one group are at a different depth in the cell-wall to those in the other group. Patient observation revealed the fact which we were afterwards able, as we shall see, to substantiate by sections of cell-walls showing the 'pits' or cavities at different levels in the wall (Pl. XVIII, Fig. 4). The explanation is that the secondary cellulose wall is deposited as a series of daily concentric laminae and the pits in these laminae are at first, in a young cell, all superimposed. Later, as the cell grows, these laminae shear or slip upon one another owing to a torsional force. A pit may be compared to a mouth-like piece cut out of a pack of cards all evenly superimposed upon one another, if the cards be slightly slipped upon one another so that their edges are no longer vertically over one another then the 'pit' in the pack will be closed, and instead of a 'pit' we shall have a series of cavities in individual cards within the thickness of the pack. Evidence of the correctness of this explanation can often be seen; pits can be found that show a series of some three or four parallel delicate dot-like appearances running across them in a direction

vertical to their long axis, these are the corners of pits at different levels that have just started to slip upon one another.

If hairs with but slightly thickened walls from the leaf of a cotton plant (we have found *Gossypium herbaceum* variety *Goghari*, a commercial cotton of the Bombay Presidency, to yield most suitable material) be mounted in water for microscopic examination and then allowed to desiccate beneath the cover slip then, upon moistening them again, short lengths of some hairs can at times be made to crack into a spiral ribbon.

The cracking of the wall of a hair into a spiral ribbon as above described, and shown in Pl. XVIII, Figs. 3 and 7, probably could only occur in a slightly thickened wall in which there were but a few concentric layers of thickening material and with their pits, if present, all super-imposed. The pits in such a cell extend into one another by spiral cracks from their corners around the cell-wall. A spiral split thus originates around the secondary wall of the cell. Such a split also occurs in a cell-wall seemingly without distal pits. In this case a crack common to all the laminae starts at a weak point and owing to torsion is carried spirally around the wall.

Flatters (15) in his Text-fig. 26, pictures a hair with a spiral split, but explains it as a fibre being enveloped by a fungus mycelium.

A point of fundamental importance is learnt from the observations just given, that is, that there are single hairs of at least two kinds upon organs outside the fruit capsule, they are—(1) hairs with basal pits only, and (2) hairs with both basal and distal pits. It is often quite impossible to say to which of these types a hair belongs, because of the oftentimes extreme difficulty of detecting the distal pits. That the two types exist, however, there is no doubt, although the second type would appear to be very much less common than the first, on all organs except the petals. The hairs are somewhat deciduous and usually break off on becoming old, but when they remain on the epidermis the distinguishing features between the two types are that at death one collapses flat and convolutes, whilst the other does neither (Pl. XIX, Fig. 38). Subsequent study of the hairs upon the seed coat will show that it is the hair with the distal pits that convolutes.

Very definite but fine and parallel line-like appearances running across the hair at an angle to the horizontal are often to be seen in the wall of an old hair. Similar lines can be found on the opposite wall-surface, but the directions of the lines on one surface intercross with those on the other. As a rule this intercrossing is only seen under the microscope on the sides of the hairs (Text-fig. 3 and Pl. XVIII, Fig. 8). This is owing to the impossibility of bringing generally the concave and convex opposite sides of a hair into one focus. This intercrossing is exactly the appearance produced by winding spirally a string around a piece of glass tubing. If on one side the string cross the tube from right to left, then on the other it will cross from left to right, and seen through the tube the directions will intersect. This fine spiral

line-like appearance is a crack in the laminae of the secondary cell-wall. It seems often to start in what was originally a pit. Presumably, however, such a delicate crack may arise at any weak point under stress. These fine cracks may be confined to a single lamina of the wall or they may penetrate a few of the laminae. Later these laminae may slip upon one another, owing to torsion during growth. This slipping accentuates the number of the cracks and their parallelism. Robinson (12) has described similar cracks in the walls of the tracheides of wood when subjected to stress.

Single hairs are common on the external surface of most varieties of *Gossypium*: Asiatic, American Upland, and Vine-Leafed (Sea Island and Egyptian). On *Thespesia populnea* and *Thespesia macrophylla* single hairs also are to be found.

(2) *The Stellate Hairs on the Plant Body.*

Stellate hairs are usually to be found upon the surface of leaves, stems, and other organs of all varieties of *Thespesia* and *Gossypium*.

Typically, a stellate hair (Text-figs. 10 and 11) consists of a group of several cells emerging from the epidermis side-by-side, but united (adhering) at their bases. This group of hairs is the result of several vertical divisions of an original single epidermal cell (Text-figs. 7 and 8). Each of the unicellular divisions of the initial cell then grows out as a ray from the basal part which is joined with its neighbours. In a stellate hair-group, the individuals composing it are always hair-like in form. Between the neighbouring cells of the tuft pits exist in their adhering basal portions, as also between each unit and the surrounding epidermal cells. The primary cell-walls of two adjacent cells persist as a lamella across the middle of the pits. There is a great deal of variation in the amount of adhesion between neighbouring cells. Sometimes this is very slight and only at the base (Text-figs. 10 and 11), as in the case of the stellate hairs on the external organs of modern commercial cottons. At other times the fusion extends slightly above the base.

Study of the stellate hair-groups show that there is a tendency for them to break up into portions consisting of a few cells or even of a solitary cell. Two-celled or bifurcate portions (Text-fig. 18) are common. This tendency for the reduction to a bifurcate form is presumably brought about by the exigencies of nutrition. Two cells may mean that a single cell tends to become a major supporter of one or two more, and the more tardy food-winner goes to the wall as it were. A single cell would be at a great advantage in having all the food material from the immediately surrounding epidermal cells. Thus reduction to two and then to a single cell perhaps arises.

In the bifurcate types of hair derived as described above there can be found all stages of fusion. Sometimes it is typically but two cells of a

stellate hair-group with the pits through the separating walls (Text-fig. 18). At other times the separating walls have gone and the branches come off from a common basal portion.

Another type of reduction to a trifurcate or bifurcate derivative of a stellate hair is also often to be found. In this one cell bears up another one or two on a short stalk, as it were, from out of the epidermal mass; like a mother-cell with daughters upon its side. (Pl. XIX, Fig. 23 is of this type of branched hair, but from within the capsule.) The daughter-cells are attached by a short bulbous basal portion to the main or mother-cell, with intercommunicating pits between the two. Sometimes there may be a single daughter-cell at right angles upon a small cell. This is to be found on *Thespesia macrophylla*.

(3) *The Peltate Scales on the Plant Body.*

The peltate scale (Text-figs. 14 and 20) is a common epidermal outgrowth upon *Thespesia populnea*. It is formed of a large number of cohering cells whose union is very complete proximally, where they form a short common vertical stalk portion. The cell units are often somewhat flattened and triangular in shape. From the stalk each unit turns horizontally outward, being still concrescent with its neighbour on either side, only a short portion at the tip being free. The result is a peltate, stalked scale. There is a considerable variety in the length of the stalk. Between pairs of adjacent cells of a peltate scale pits occur in the common or party-wall. These pits can be readily found in the proximal half of the cells. They also occur in the more distal portions, but their detection here is more difficult. They are more readily to be seen in young stages with only partially thickened walls than in old forms with fully thickened walls (Text-fig. 17). The walls of the individual cells eventually become greatly thickened. It will be seen in what follows that the peltate scale gives rise to other forms of organs. The term 'peltate scale' will be restricted to organs whose individual components have a short, more or less vertical portion which soon turns horizontally so as to run parallel to the epidermis, the scale being circular in outline with the stalk or point of attachment at the centre.

The whole lower surface of the leaf of *Thespesia populnea* is covered with typical peltate scales (Text-fig. 20), and they abound upon the wall of the ovary. There are peculiar depressions, or cavities, upon the under side of the leaf of this plant (Text-fig. 19). They occur at the junction of the main veins, where these latter branch off from the leaf-stalk. Such a cavity extends backwards for a short distance between the junction of each two contiguous veins and then ends blindly. It is about half a millimetre deep, and is lined on its free surface by a palisade epidermis (Text-fig. 20). The surface of the cavity is covered with peltate scales, and the outer wall of the

epidermis is in places thickened by delicate linear ridges so as to produce surface striations, like those sometimes to be seen at the base of a hair in *Gossypium* (Text-fig. 4).

The bases of the peltate scales, both within the cavities and upon the general surface of the leaf, are sunk in a depression of the epidermis, and this depression is frequently situated over a mucilage cavity in the leaf tissue (Text-fig. 21).

The petals of *Thespesia populnea* show upon their lower surface a most remarkable series of transitional forms of these scales. The typical peltate scale, with its constituent cells all radiating from their centrally situated bases, is abundant (Text-fig. 14 and Pl. XIX, Fig 19).

There is a tendency, however, for the constituent cells to become longer than is the case with the scale upon the leaves. In the larger scales thus formed the ray cells have a longer free portion at their tips than the smaller ones upon the leaves, and they show an inclination to split up radially into groups of a few, especially of two, cells. A further interesting point is that as the cells become longer not only does their diameter become less, and their shape less fusiform, but their walls also become thinner. The daily deposition of cellulose has now to be laid down by the protoplasm upon a larger area. No peltate scales as such have been found upon *Thespesia macrophylla*, nor upon any variety of Gossypium, but vestiges of these scales do occur in various forms.

The peltate scale has in its history undergone modification, and this can be traced on *Thespesia populnea*. Such modification has proceeded along one of two lines. In one case it is accompanied by the squeezing out, as it were, of some of the rays into the inner portion of the rosette (Pl. XIX, Fig. 25). These squeezed-out rays may eventually disappear in the evolutionary history. In some related groups of the Sterculiaceae they persist as an inner tier of rays, thus resulting in a double-storied peltate scale. The outcome of this process is to produce a derivative of the peltate scale which has fewer rays. This form is usually somewhat eccentric. Often the rays of these forms are coherent in pairs (Pl. XIX, Fig. 24).

The second line of modification is represented by forms, especially to be found on the distal portion of the petal, which have largely lost their circular symmetry, the constituent cells on one half having developed to a much greater extent than the cells on the other (Pl. XIX, Fig. 20). The penultimate expression of this one-sided growth is to produce a flat shield-like plate of cells with their points of insertion at one extremity of the shield and their tips at the other (Pl. XIX, Fig. 21).

Ultimately from the shield-like plates single cells have separated out. The method of evolution of the single cell from the plate is easily followed. It corresponds with the evolution of a single cell from a stellate hair-group, as already described. Stages of the association of two or three cells are

common, and the final isolated hairs (Pl. XIX, Fig. 22) occur in abundance, especially at the base of the petal. Their structure has already been described and figured (Pl. XVIII, Figs. 1 and 2).

Upon the petals of *Gossypium Stocksii*, in addition to typical stellate hair-groups, many groups can be found with their individual cells coherent for a distance above the base (Text-fig. 12). The hairs may be joined for about as much as a third of their length. They may represent a stage in the evolution of the peltate scale. A bifurcate hair (Pl. XIX, Fig. 37) evidently derived from these organs, also is common. Amongst these organs there are also to be found upon the petals cases of two long hairs cohering side by side for some distance (Pl. XIX, Fig. 37). Similar hairs occur upon the petals of *Thespesia macrophylla*. These hairs have pits in their party walls. (Pl. XVIII, Fig. 9, is of a similar form from within the capsule.) They are obviously derivatives of a peltate scale.

(4) *The Club-shaped Bodies on Vegetative Organs.*

Multicellular club-shaped epidermal outgrowths are found on all forms of Gossypium, on *Thespesia macrophylla*, and on *Thespesia populnea*.

On *Gossypium Stocksii* at the base of the petals there are large numbers of simple multicellular hair-like bodies. They each consist of a tier of cells vertically projecting from the general epidermal level. There may be some twelve or so cells in all. The distal cell forms a dome-shaped termination or cap to the structure. Vertical walls are few and are not laid down in any regular order, frequently they are absent altogether. This type of body is common amongst the Malvaceae, similar bodies, for instance, can be found on almost any species of the genus *Hibiscus*. It is a developmental stage of the older form of this body found on the leaves, both floral and vegetative, of *Gossypium Stocksii* and on *Thespesia macrophylla*. The fully developed body (Text-fig. 27) is spherical, owing to a development of cell division by vertical walls. Excepting the proximal two or three cells, and the distal one, all others show considerable propensity to increase in number. The proximal cells form a short stalk, and the distal one a cap.

The development of this body has been followed in the case of *Gossypium* and *Thespesia*. In *Gossypium cernuum*, an Asiatic cultivated cotton geographically isolated in a region east of the Brahmaputra River in Assam, this body arises by the outgrowth of a single epidermal cell (Text-figs. 23, 24, 25, and 26). When this has attained a height above the neighbouring cells about equal to that of its embedded portion it divides into two by the formation of a transverse wall, further transverse walls following in succession. After the formation of the first two or three proximal walls the more distal ones form closer together.

Vertical walls now occur in these distal cells except in the terminal one (Text-fig. 27). The terminal cell remains undivided as a cap cell. It is

preferred to call these bodies simply club-shaped bodies, and not glands as they have been termed by some authors.

In *Thespesia populnea* batteries of the club-shaped bodies are to be found on the under side of the leaf pulvinus at the junction of lamina and petiole. There is nothing in this case to mark their position to the naked eye. In *Thespesia macrophylla* a very obvious slit-like depression (Text-fig. 22) on the under surface of the main vein of the leaf contains an aggregation of them.

Like the simple and stellate hairs, the club-shaped bodies occur on the leaves, especially above the veins or the vein endings. On cottons they are common on the leaves and bolls. On the main vein, on the under surface of most types of cotton leaves, a pit-like depression is found containing a group of these bodies. Upon the secondary vein on each side of the midrib there may also be a smaller collection of them. In any type of cotton they may, or may not, be present in different plants. Genetic investigation shows the presence, or absence, of these depressions, or 'leaf glands', to be inherited characters behaving as an allelomorphic pair in a regular type of Mendelian inheritance.

No direct evidence has been obtained as to the function performed by these club-shaped bodies. They have been regarded as glands by some, and it has been suggested that they secrete a substance attractive to insects. Insect visitation to the batteries of them upon the leaves has not been observed.

Around the base of the stalk of each club-shaped body is a delicate groove. The surface of the surrounding epidermal cells shows numerous fine striations radiating from this groove. The same thing has been noticed already as occurring at times at the base of a hair.

If these club-shaped bodies secrete anything it is probably water. They, like the trichomes already referred to, are, however, just as likely to be water-absorbing organs. 'It is, in fact, impossible to draw any sharp distinction between secretory hydathodes and water-absorbing trichomes. After a certain stage in the life of a leaf the hydathodes not infrequently devote themselves exclusively to absorption' (Haberlandt) (7).

Upon the calyx of the Gossypia, at the base and alternating with the three bracts, there are three distinct triangular patches of the club-shaped bodies. These are to be regarded as batteries of the club-shaped bodies upon the three fused sepals of the gamophyllous calyx. Sometimes above these, and alternating with them on the calyx, there may be smaller patches of these bodies. These patches of club-shaped bodies on the calyx are often spoken of as nectaries. They certainly are more actively secretory than the patches on the leaves, for a suspicion of moisture can be seen upon them at times. They ordinarily have a reddish, or yellow, colour, and a shiny appearance.

PART II.

THE EPIDERMAL OUTGROWTHS WITHIN THE CAPSULE.

There are two places within the fruit capsule of the subjects of this study where epidermal outgrowths may be found. These occur firstly on the sterile placentae as swellings representing the regions of the fused margins of the carpels; and, secondly, on the seed coat.

In *Thespesia macrophylla*, *Gossypium Stockii*, *G. brasiliense* (Kidney Cotton), and *G. hirsutum* (American Upland Cotton), such organs can be found on the sterile placentae. On Marie Galante, a Sea Island type of cotton, comparatively long hairs may occur in this position. In many cottons, however, there are no epidermal outgrowths within the capsule, except upon the seed-coat. The tendency in the Hibisceae would seem to be for the epidermal outgrowths within the capsule to restrict themselves to, or be preserved upon, the seed coat.

The result of the studies herein described shows that we can group the epidermal outgrowths within the capsule in the same four categories as we did those on the vegetative organs. The order there followed, of describing first the single hairs, and then the more complex organs, was adopted because it was the order in which we studied them, and because it seemed a logical method of progression in our work.

It is intended now to describe these four classes of outgrowths in the reverse order to what was done in the case of the similar organs upon the vegetative parts. The reason for this will presently become apparent.

We will consider the epidermal outgrowths within the capsule in the following order then:

I. The Club-shaped Bodies.
II. The Peltate Scales, and their derivatives.
III. The Stellate Hairs, and their modifications.
IV. The Single Hairs.

I. *The Club-shaped Bodies within the Capsule.*

The club-shaped bodies found upon the vegetative parts also occur at times within the capsule, both upon the sterile placentae and the seed coat, but they have only been found here in a few forms. They are not common. *Gossypium Stocksii* and Ashmouni cotton produce them on the sterile placentae. *G. neglectum* var. *roseum*, and *Thespesia populnea*, have yielded them upon the seed-coat. The chief interest in the club-shaped bodies within the capsule is the change in form effected by the altered environment. Rarely is the normal form on vegetative parts found. There is a considerable tendency for vertical cell-walls not to be formed, and for the bodies to become somewhat elongated and hair-like. The cell-walls

apparently always remain thin. A peculiar modification of the simple body found in *Gossypium Stocksii* capsules is shown in Text-fig. 28, also one from the same organ of Ashmouni cotton is seen in Text-fig. 29.

II. *The Peltate Scales and their Modification within the Capsule.*

The peltate scale, as already mentioned, is not usual upon the vegetative parts of the plants which are the subject of our study. *Thespesia populnea* being the only plant upon which this body, recognizable as such, has been found.

The study of this scale, and of the modifications that it undergoes, has yielded some remarkably interesting facts.

The hairs upon the seed coat of *Thespesia populnea* are polymorphic, and objects of great beauty when viewed under the microscope. They furnish an extremely interesting series of forms showing all stages in the evolution of a single hair from a peltate scale. That is to say, they illustrate the development of a simple single organ from a more complex. We have seen this same process at work on the leaf. It is well, however, to point out at once a fundamental difference between a fully grown single cell derived by a process of reduction from a peltate scale on an external surface, and one so derived within the capsule. In the latter case the cell-wall is comparatively thick, whilst in the former it is thin. We shall return to this difference in the final discussion. The series of stages by which this development has taken place is well illustrated in the photomicrographs Pl. XIX, Figs. 14, 15, 16, 17, and 18.

The typical peltate scale with many fused units, as found upon the vegetative parts, is not found within the capsule. There is to be found, however, a very obvious simplification of it, which consists of a form with many fewer component cells. The constituent cells, however, do not bend so as to run parallel to the epidermis. Forms with four fused component cells are common (Pl. XIX, Fig. 14). The cells when mature have extremely thick secondary cellulose walls. They are, in outline, shaped somewhat like a triangular vase with the body adherent to its neighbour on either side, and a short free projecting neck. Very obvious transverse pits occur in the walls of adjacent cells. Three-celled (Pl. XIX, Fig. 15) and two-celled (Pl. XIX, Fig. 16) aggregations can also be found in numbers, and the reduction to a single cell (Pl. XIX, Fig. 18) can be followed very easily, for pairs with one large cell and a dwindling second cell are abundant (Pl. XIX, Fig. 17).

This reduction to a single cell is accompanied by a change in form and an increase in the size of the persisting cell. The single cells thus derived always have very thick walls. Their walls are much thicker than those of any individuals of a stellate hair-group. When fully grown these hair-cells

are about 1 mm. long. Their walls, at first thin, soon start to thicken from day to day as they grow older.

The position and presence of pits and spiral markings in the walls of the units of peltate scales both when fused together, and when existing as solitary hairs, are of especial interest.

In a group of two, three, or four cohering cells, pits in the party-walls are often very plainly to be seen (Plate XIX, Fig. 14) along the line of juncture. They cannot ordinarily be seen in single cells, but can be found in sections of the wall. The individual cells often end distally in a narrower finger-like portion. In the walls of this portion there may at times be seen spirally arranged fine crack-like lines such as we have already described in the case of cells on vegetative organs. These, for instance, are shown on the tip of the longest cell of the four in Pl. XIX, Fig. 14. A photo-micrograph at a higher magnification of this is given in Pl. XIX, Fig. 33. The concentric laminae making up the cell-wall usually show very plainly in these cells. We have succeeded in cutting sections by means of a microtome to illustrate the structure of the cell-wall. Pl. XVIII, Fig. 10, shows a longitudinal section across the proximal region of a two-celled derivative of a peltate scale. Pl. XVIII, Fig. 4, is the portion of the wall seen in Pl. XVIII, Fig. 10, photographed under high magnification. It shows the thin outer primary wall, and the thick secondary cellulose wall made up of a series of concentric deposits as described in the case of the cotton hair by Balls (9). It also shows the pits buried at varying depths within the different concentric layers of the cell-wall. The pits are no longer horizontal but oblique or often vertical, and they occur all over the cell-wall, and are not restricted to the cohering parts of the two cells. It is of interest to note that in the entire hair when the pits have slid away from over each other in the different laminae they become invisible under the microscope, but can be found in sections. This is probably in large part due to the refraction of the cellulose wall.

Another important point can be understood from these observations—the cause of the reduction of the many-celled peltate scale to single-celled units. As the units of a peltate scale grow they are subject to a considerable torsion due to the phenomenon of nutation produced in the hair by the daily movement of the sun. This torsion, or twisting around the vertical axis of the cell, as we have already explained in the case of the hairs on vegetative parts, causes a slipping of the concentric layers of the cell-wall in a plane parallel to the outside of the cell. The pit in one concentric layer thus is no longer opposite to the corresponding pit in its neighbours, owing to the pit cavity in one layer having moved beyond that in another. In this way the passing of nutrition from one cell to another becomes arrested, for the pits originally passing in one line through all layers cease to 'track', and if for any reason one cell is unable to obtain its

food by its basal pits it will atrophy. Thus the reduction of a peltate scale to single-celled units is primarily due to torsion. Further evidence of the result of this torsional force upon the cell is seen in the spiral arrangement of the fine cracks in the free extremities of the cells. Often also in many two-celled forms the individual cells show quite a twisted appearance at the concrescent common base (Pl. XIX, Fig. 16).

Such a series of hairs, demonstrating so beautifully the evolutionary history of the short thick-walled hair, does not exist upon the seed of any other of our types. The final product, the short thick-walled hairs of limited growth in length, however, are found upon the seed of many cottons constituting the layer known as 'fuzz', and is well seen when the layer of longer hairs known as the 'lint' is removed.

This short thick-walled hair of limited growth in length is then the second type of single hair that we have seen to be evolved from a peltate scale. The first type was a longer hair with a thinner wall whose evolution took place upon the external surface of the plant, and which we have fully described and figured in the case of *Thespesia populnea* in the first part of this paper. Both types of hairs on account of their origin have 'pits' in the laminae of their secondary walls, and in both types owing to torsion these pits tend to become obscured owing to the shearing, or slipping, of the laminae upon one another. The longer hair within the capsule does not commence to thicken its wall from the beginning, but waits until it has completed its growth in length.

In *Thespesia populnea* the longer thinner-walled hair does not seem to have entered the capsule. The type of hair found there is the thick-walled one.

In *Thespesia macrophylla* a pair of long fused hairs such as have already been described as occurring upon the vegetative parts of this plant and of *Gossypium Stocksii* (Pl. XIX, Fig. 37) can be found amongst the hairs borne within the capsule upon the sterile placentae. Their common walls are characterized by many pits (Pl. XVIII, Fig. 9). These cells represent a vestige of a peltate scale.

Within the capsule of cultivated species of cotton plants the longer thinner-walled hair occurs upon the seed-coat in abundance, and constitutes the layer known as 'lint', or the cotton of commerce.

The frequent occurrence of branched forms amongst the lint hairs of cottons is explicable by their phylogenetic origin from a group of coherent cells such as the peltate scale. The lint hairs of most cultivated and wild cottons will, if patiently searched through, show this phenomenon of branching. Sometimes the 'branch' is separated from the main hair by a common pitted portion of the wall, at other times there is no septum between the two cells. The breaking down or absorption of the separating pitted wall is necessary, or torsion would close the pits by slipping them

away from each other in the different laminae, and so cut off nutrition from the branch cell. Two fused cells, whose contents were at first only separated from one another by the middle lamella across the pits in their party-walls, often show when old the wall of one cell to be much thinner than that of the other. The cell with the thinner wall is the branch. This is because of a shearing of the laminae and a consequent difficulty for the branch cell to obtain its necessary requirement of nutrition which results in the deposition of its cellulose wall being checked. Not only may a branched cell have as one of its 'branches' a vestige of a small cell from the upper tier that ancestrally lay over it in a double-storied peltate scale on a radius parallel to its own (Pl. XVIII, Fig. 6), but it may have a 'branch' which was ancestrally a cell on another radius diametrically in a line with itself (Pl. XVIII, Fig. 5). Various forms of branching (Pl. XIX, Figs. 34, 36, and Pl. XVIII, Figs. 5 and 6) of hairs are explicable on the idea of origin from a single- or double-storied peltate scale. Single cells derived from a peltate scale frequently show a cone-shaped foot portion, evidence of an ancestral wedging with other cells in the base.

Upon the sterile placentae of *Gossypium Stocksii* there occur many hairs with a somewhat boot-shaped foot (Pl. XIX, Fig. 30). These hairs show scattered pits throughout their length and their walls are at first thin but eventually thicken. These hairs furnish exceedingly good material upon which to begin the study of the pits (Pl. XVIII, Fig. 11). Upon them the pits can be found with certainty by one who has not seen them before, and after which one is enabled to recognize them under more difficult conditions. The boot-shaped foot was at first a puzzle, for although the wall with its pits and its later thickening agreed with the characters of a derivation from a peltate scale, we could not reconcile the boot-like base with the usual conical one.

Upon further study, however, we found forms with vestiges of other cells fused, usually one, at other times two, with the basal portion of a hair (Pl. XIX, Fig. 32). These cells differ from the surrounding epidermal cells in having thick walls. Sometimes the base of the hair and these cells only communicate with one another by means of pits in their walls; more often the separating walls have gone, giving rise to the toe-like projection of the base of an apparently single cell. At times the main cell shows a spur-like branch higher up than the base (Pl. XIX, Fig, 31).

We have now discussed the origin and variations of two types of hairs to be found upon the seed-coat of species of *Gossypium* and *Thespesia*. It is only upon the seed-coat of the former that the two types may both occur.

It may be well to summarize carefully here some of the characters of these two types of hair, known commercially in the case of cotton as 'fuzz' and 'lint'. Their structure and relationship to one another have been the subject of considerable investigation by many workers. Balls (9) came to

the conclusion 'that the so-called fuzz hairs are analogous with the lint hairs'. The two types of hairs are more than analogous, they are homologous, i. e. similar in origin and in structure. Due to their similarity in origin, both lint and fuzz hairs have pits in their walls. In both hairs as they grow old these pits become obscured by the slipping of the wall laminae upon one another. The difference between fuzz and lint hairs is but in degrees of length and wall thickness.

The geneticist in his work upon the cotton plant has for long been puzzled to know what, if any, were the relationships between 'fuzz' and 'lint'. We can now say that both 'fuzz' and 'lint' hairs have evolved from the same thing—the peltate scale, but along slightly different paths. It is interesting to see how these different lines of evolution have produced a different result in each case.

The 'fuzz' hair is a cell of limited growth in length, whose walls early start to thicken by the daily deposition by the protoplasm of a shell of cellulose on the inside of the wall. By the time some twenty or so layers have been deposited (Balls (8)) the cell-wall has reached its maximum growth in thickness, and the cell cavity and its contained protoplasm have become diminished, and the cell dies.

The 'lint' hair is a cell which continues to grow in length for some twenty days or so, during which time little or no increase in thickness of its wall takes place. It is, then, after this period of growth in length, that the cell starts a daily deposition of cellulose on the inside of its wall, and deposits some twenty or so concentric shells of cellulose before it dies.

These differences between the ontogeny of the two hairs are directly explicable by their phylogeny.

In the phylogeny of the hair of limited growth in length (i. e. the 'fuzz' hair) we have seen how—(I) the young thin-walled cells constituting a peltate scale inside the capsule of *Thespesia populnea* become (II) thick-walled, contemporaneously with their growth in size, and, how (III) the constituent cells with already thickened walls next separate into solitary individuals.

In the phylogeny of the longer type of hair (i. e. the 'lint' hair) we have also seen how on external organs—(1) the young thin-walled peltate scale gives rise to (2) thin-walled shield-like plates of cells, which (3) break up into thin-walled single-celled units, and (4) these thin-walled units grow by apical growth into long hairs, which (5) then thicken their walls by concentric deposits of cellulose.

Now we understand why the lint hair of the cotton seed waits for a period of some twenty to twenty-five days before it starts to thicken its wall (Balls (8)), whilst the fuzz hair does not so wait. This delay in the ontogeny of the lint hair represents the period occupied by stages (2), (3),

(4), above in its phylogeny, which stages have never occurred in the evolution of the fuzz hair.

Graphically we can summarize the evolution of each hair in the following way:

The Evolution of the Fuzz Hair (Pl. XIX, Figs. 13, 14, 15, 16, 17, and 18).

I. Thin-walled young peltate scale. → II Thick-walled mature peltate scale. ↓ III. Thick-walled solitary single-celled units (fuzz hairs).

The Evolution of the Lint Hair (Pl. XIX, Figs. 19, 20, 21, and 22).

1. Thin-walled young peltate scale. → Thick-walled mature peltate scale.
2. Shield-like plates of cells with thin walls. → Shield-like plates of cells with thick walls.
3. Thin-walled cells break apart into solitary cells.
4. Thin-walled cells grow in length.
5. Thick-walled long hairs (mature lint hairs).

III. *Stellate Hairs and their Modifications within the Capsule.*

The typical multitufted form of stellate hairs is not so generally found within the fruit capsule as it is upon the external epidermis. It, however, does occur at times. On the micropylar end of the seed of *Thespesia macrophylla* it is common, and also it occurs less frequently upon the seed-coat of *Gossypia*. *Gossypium Stocksii*, *G. cernuum* and *G. neglectum* var. *roseum* generally yield it. The tendency for the stellate hair-groups to break up into forms with a fewer number of rays or single cells is present within the capsule just as we have seen it to be on external surfaces. This reduction often commences by the bulbous bases of some of the cells in a stellate hair-group being borne up by one of their neighbours so that what may be called a parent-cell arises with one or more daughter-cells upon its walls (Pl. XVIII, Fig. 12, and Pl. XIX, Figs. 23 and 35). The cavities of the daughter-cells are still only separated from the cavity of the parent-cell by

pit-membranes (Pl. XIX, Fig. 27), although the daughter-cells have now, of course, lost their connexion with other cells. Where the daughter-cell comes off from the main or mother-cell the latter is often enlarged into a knee-joint-like swelling. Single hairs (Pl. XIX, Fig. 28) can at times be found with these knee-joint-like swellings, and at this point one usually finds a group of pits. Their meaning now is evident. The group of pits in this case it is to be noted is solitary. The origin of these pits is different from the pits on the hairs derived from the peltate scales. They are basal pits in the case of the daughter-cell carried up away from the base, but in the wall of the main cell they are secondarily formed. Hairs with knee-joint-like swellings, which sometimes show the pits, can be found upon the seed-coat of many cottons. Such hairs and others derived from a stellate hair-group generally show their ancestry by possessing a bulb-shaped foot. Sometimes a daughter-cell may be borne up by the parent or main cell for a considerable distance, the two thus branching away as it were from the top of a common foot-like portion. The separating walls may entirely disappear so that an apparently furcating single 'cell' results. A phenomenon of frequent occurrence within the capsule upon the sterile placenta and more rarely upon the seed-coat is the fusion by their bases of two cells of a stellate hair-group. Here, again, the common pitted portion of the cell-walls may persist or disappear. The type of bifurcate 'cell' that results in this way is especially common upon the sterile placentae of *Gossypium Stocksii* (Pl. XIX, Fig. 34) and Marie Galante cotton.

IV. *The Single Hairs within the Capsule.*

We have already traced through their evolution from a peltate scale to their present day form two types of single solitary hair. One of these hairs, the hair corresponding to the 'fuzz' hair of cotton (Pl. XIX, Fig. 18), has developed within the capsule. It is only within the capsule of *Thespesia populnea* that we have found the actual stages of its evolution, the genus *Gossypium* apparently having come off from the ancestral stock after that stock had given rise to the single hair. That stock was not necessarily *Thespesia populnea*, but was at least closely related to it.

The second of these hairs whose evolution we have traced is that corresponding to the 'lint' hair of cotton (Pl. XIX, Fig. 22). This hair we have seen also arose from a peltate scale, but its origin first took place outside the capsule upon the floral organs of such a form as *Thespesia populnea*, it only entering the capsule in the evolution of *Gossypium*, after it had attained the main details of its present structure upon some ancestral form. All epidermal outgrowths, including the club-shaped bodies, find an environment within the capsule especially suited to growth in length.

The evolution of the 'fuzz' and 'lint' hairs as already given explains many details in their structure that before were inexplicable. These two

types of hair are by far the most common and usual upon the seed-coat of *Gossypium*.

In addition to the 'fuzz' and 'lint' types of hair within the capsule a third type of single hair is found as a rare occurrence in cotton, and *Thespesia macrophylla*. This is a single hair with a knee-joint-like swelling along its course, and which may show pits upon its wall at the swelling (Pl. XIX, Fig. 28). The evolution of this type of hair has already been traced when dealing with the stellate hairs within the capsule.

We now have to describe a fourth type of single hair found within the capsule of some forms. This is a single hair with a bulbous root (Pl. XIX, Fig. 29). It is common upon the seed-coat of *Thespesia macrophylla*, and it can be found amongst the hairs on the seed-coat of most types of *Gossypium*. Indeed, it has often been described and figured in treatises upon commercial cotton, but its structure and meaning have never been understood. Typical figures of it are given by Flatters (15) in his plates of Florida, Brown Egyptian, New Orleans, Peruvian Rough, and Hingunghat, cottons.

The characteristics of this hair are its bulbous base with pits, but without pits more distally upon the cell-walls; the markings shown in the photograph are not pits, but the spiral cracks due to torsion, which are frequent in this type of hair. The wall becomes greatly thickened, and on death remains spherical in transverse section, and does not collapse flat as does the wall of a typical lint hair. Furthermore, at death it differs from a typical lint hair of *Gossypium* in that it is not thrown into the spiral convolutions, or twists, so characteristic of the typical lint hair. There is little difficulty in recognizing this hair as a solitary representative of a one-time stellate hair-group.

In *Gossypium cernuum* and *G. neglectum* var. *roseum* (both very coarse linted forms of Old World cottons) this hair is comparatively common upon the seed-coat. These two cottons are known commercially as types yielding upon ginning a comparatively high percentage of cotton to seed, and this high 'ginning percentage' is directly related to the presence of this type of hair upon the seed. Much attention has been given by geneticists, working upon cotton, to the inheritance of 'ginning percentage', and these observations now show that this property may be a complicated character due to the presence of two types of hair in addition to any 'fuzz' upon the seed-coat, and not one involving a single factor in inheritance.

There is one more case of peculiar hair to be described, and that is the hair upon the seed-coat of *Gossypium Stocksii*. The seed of this cotton is covered with a felt of short brown hairs. These differ from the hairs of commercial cottons in that they never grow long enough to spin, when mature their length is but some 8 mm. long. They have no distal pits upon their walls, and on death these hairs do not convolute as do the hairs

upon the seed of other cottons. They may show an occasional twisting at intervals, but this has nothing resembling the regular convolution found in commercial cotton, and is not the same phenomenon at all. These hairs would appear to be derived from the bifurcate type of derivative of the possibly evolutionary form of the peltate scale (Pl. XIX, Fig. 37) common upon the petal of this wild cotton. In this bifurcate hair fusion is approximately confined to the base, and hence the single hair derived from it would show no distal pits.

There is no hair upon the seed-coat (although there is upon the sterile placenta) of *Gossypium Stocksii* representing either true lint or true fuzz. *G. Stocksii* is to be regarded as a side line from the origin of the Old-World *Gossypium* stock. It is primitive, but not in the direct line of descent of present-day Asiatic cottons.

General Observations.

It has been shown that the solitary hairs upon the epidermis of species of the genera *Thespesia* and *Gossypium* are derivatives of either stellate hair-groups or of peltate scales. Differences in the structure of these hairs and some of their complicated features are explicable on an understanding of their origin. The evolutionary histories of the stellate hair-groups and of the peltate scales themselves remain to be traced. It is probable that one of them is derived from the other, but if so this evolution would seem to have taken place in the ancestral line of our types, prior to their origin. Stellate hair-groups and their derivatives, and peltate scales and the derivatives from them, behave as independently inherited organs on the plants where they occur. Mutant forms of *Gossypium* with no stellate hairs or their derivatives are known. Such forms of Asiatic cottons are growing at the present time in the experimental plots attached to our laboratory. The Egyptian 'Hindi Weeds' would appear to present a similar phenomenon amongst the New-World cottons. Such mutant forms have no hairs upon their vegetative organs, which glabrous condition is to be accounted for by the total absence from the plant of the stellate hair and its derivatives. The absence of the stellate hair is correlated with small petals, which present a very unusual form when in the bud. This glabrous mutation is an inheritable condition. Upon the seeds both fuzz and lint hairs may be present. Upon the seed-coat of *Thespesia populnea* alone, developmental stages of the hair homologous with the fuzz of cotton are found. The only hairs found within the capsule of *Thespesia macrophylla* are the stellate hairs and their derivatives. In *Gossypium* the fuzz and the lint hairs on the seed-coat behave in inheritance as organs quite independent of one another. Both may or may not be present, although the total absence of the lint is apparently a very rare condition, whilst on the other hand fuzzless-seeded types are common. The independent behaviour in inheritance of the presence, or

absence, of these two hairs upon the seed-coat is explained by the fact that although evolved both from the same thing, the peltate scale, their paths of evolution have been quite independent. There were no doubt forms in the ancestral line of the cottons both with and without peltate scales upon external organs and inside the fruit capsule, just as we have *Thespesia populnea* with them, and *Th. macrophylla* without them. The fuzz hair has evolved within the capsule from a constituent cell of a peltate scale, after its cell-walls had become thickened. The lint hair, on the other hand, evolved from a peltate scale before its constituent cells had thickened their walls. This evolution took place externally on the floral organs, and it was only after its evolution to the stage of a single cell that the lint hair appeared within the capsule.

A study of the capsular fruit in the Malvaceae, and in the nearly related Bombacaceae and Sterculiaceae, shows that it and the schizocarpic fruit known sometimes as a carcerulus, which is so characteristic of many of the Malvaceae, are really formed on the same plan. They have both arisen by the fusion of several separate monocarpellary fruits. This condition of separate monocarpellary fruits occurs in some of the more primitive Malvaceae, as, for instance, *Malope*. It was when this fusion occurred in the ancestral line of a stock common to the Hibisceae, Bombacaceae and Sterculiaceae, that hairs that had developed upon external surfaces became occluded in the capsule. *Bombax*, which yields the kapok fibre of commerce, has these hairs springing from the capsule wall. *Eriolaena* (Sterculiaceae) has hairs within the capsule. In this way one can account for the presence of hairs in some cottons upon the placenta-like swellings which represent the point of fusion of the carpel margins. Once within the capsule the hairs have spread to the ovules, upon the wall of which they found an environment especially suitable to growth in length. The long hair that has given rise within the capsule of *Gossypium* to the lint hair could only have evolved from a peltate scale in the recesses of a rosette of leaf-like organs such as we have in a flower. There is no tendency for the peltate scale upon freely exposed surfaces to become eccentric. The eccentric condition arises as the result of the greater influence of light upon one half of the circular peltate scale than upon the other.

Summary.

1. The epidermal outgrowths of the two genera *Thespesia* and *Gossypium* are studied. A schematic arrangement of the Old-World and New-World types of *Gossypium* is given, so that the relationships and characters of forms mentioned can be seen.

2. The epidermal outgrowths upon external surfaces fall into four classes:

(i) Single unbranched hairs which are situated over vascular bundles. The walls of these increase in thickness by the daily deposition of a lamina of cellulose by the protoplasm. These laminae may crack, and slip independently upon each other, under torsion during growth. All single hairs have pits in their walls at the base, which pits communicate with neighbouring epidermal cells. Two types of single hairs are to be found, those with pits in their walls distal to the base, and those without such distal pits. At first a pit in one growth lamina opens into corresponding pits in the other laminae, but this common cavity becomes lost when the laminae shear, or slip, on one another.

(ii) Stellate hairs, which consist of tufts of several cells fused only at their bases, and coming off from one spot. These groups of cells tend to break up into portions of a few or a single cell. Sometimes two cells fuse to produce a bifurcate cell.

(iii) The peltate scales are formed of a number of cells fused throughout their length. They have a short vertical stalk and a main portion of the scale lying horizontal to the epidermis from which it springs. They are frequently situated over a mucilage cavity. Between the individual cells pits exist in their walls throughout their length. Upon the petals of *Thespesia populnea* metamorphic forms occur showing a series of stages from a concentric peltate scale, through a shield-shaped plate of cells to single cells. These single cells show pits upon their walls.

(iv) The club-shaped bodies are multicellular when mature, and occur on the surface especially over the veins. Aggregations of them in pit-like depressions are found on the veins of cotton leaves, and batteries of them are found on the calyx.

3. Epidermal outgrowths of the same type as the above, and derivatives from them, are found within the capsule, both upon the wall and upon the ovules. They are best studied in the reverse order to the above.

(i) The club-shaped bodies are to be rarely found within the capsule, but when they occur they find the environment such as to produce elongation in length.

(ii) The peltate scale within the capsule has split up and eventually become represented by single hair-like cells. Their evolution is well seen in *Thespesia populnea*. These single cells increase the thickness of their walls from their beginning by the daily deposition of a lamina of cellulose. Pits occur in the secondary walls from the inside of the cell to the primary wall. In old cells these pits are lost to sight by the shearing of the laminae upon one another, but can be found in sections. These cells are homologues with the 'fuzz' hairs on the cotton seed. The long single-celled hairs,

derived from a peltate scale upon the petals of *Thespesia populnea*, have homologous forms within the capsule of *Gossypium*, which are the lint hairs. These lint hairs grow long, and do not begin to increase the thickness of their secondary wall until they are some twenty days or so old. This delay in their wall-formation in their ontogeny represents the period in their phylogeny during which they evolved through a shield-like plate stage.

The frequent occurrence of branched forms amongst the lint hairs of cotton is explicable by their phylogenetic origin from a group of fused cells, such as the peltate scale. The lint hairs and fuzz hairs are similar in origin and in structure. The difference between them is but in degrees of length and wall thickness.

The fuzz hair has developed within the capsule from a thick-walled cell of a peltate scale, which cell has become solitary.

A lint hair has developed phylogenetically on the petals by the separation of a thin-walled cell unit from a developing peltate scale, which cell then thickened its wall, and later became occluded within the capsule.

A graphic representation of the evolution of these two hairs is given in the text.

(iii) The stellate hairs are not common within the capsule, but may at times be found. These tufted hairs tend to break up into single-celled units. Branched forms of stellate hair derivatives sometimes may be found.

(iv) The chief single hairs within the capsule are the fuzz and lint hairs, and their homologues, whose derivation from a peltate scale has already been described.

A hair with a knee-joint-like swelling, derived from a cell of a stellate hair-group is found as a rare occurrence.

A hair with a bulbous root, with basal, but not with distal pits, and which does not convolute, may be quite common in some types of cotton.

The hair upon the seed-coat of *Gossypium Stocksii* is in many respects peculiar. It is probably derived along a somewhat different path from the lint hair of other cottons.

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EXPLANATION OF PLATES XVIII AND XIX.

Illustrating Dr. W. Youngman's paper on The Epidermal Outgrowths of the Genera *Thespesia* and *Gossypium*.

The figures are direct photomicrographs of the object under the microscope, except figure 4, which has been obtained by projection. Unwanted hairs have been blocked out from some photographs, but in no case has the object shown been retouched.

PLATE XVIII.

Fig. 1. *Thespesia populnea*. Two hairs coming off from the same point on the base of the petal. The larger shows pits in its wall. × 96.

Fig. 2. *Thespesia populnea*. Portion of another hair as above. × 300.

Fig. 3. *Gossypium herbaceum*. Hair from the leaf, split into a spiral. × 300.

Fig. 4. *Thespesia populnea*. Longitudinal section of the wall of the hair between the two lines shown in Fig. 10, seen under high magnification obtained by projection. The picture shows

the laminae in the secondary wall, and the edge of the primary wall (extreme right). Four 'pits' (*a*, *b*, *c*, *d*) are seen at different levels in the wall. × 840.

Fig. 5. *Gossypium Stocksii.* A bifurcate hair from the sterile placentae within the capsule. × 48.

Fig. 6. Marie Galante Cotton. Portion of a branched hair from the sterile placentae within the capsule. The hairs show many pits, some are seen in surface view distally, and on the right contour, opposite the branch, others are seen in profile. × 300.

Fig. 7. *Gossypium Stocksii.* Two hairs from the sterile placentae within the capsule. The left hair has split into a spiral. × 96.

Fig. 8. *Gossypium herbaceum* var. *Goghari.* A hair from the leaf. Fine spiral markings (shear cracks) are seen in the left contour. It was only possible to focus sharply one contour at a time. × 300.

Fig. 9. *Thespesia macrophylla.* Portion of a double hair from the sterile placenta. This hair is of the same form as that shown upon the petal of *Gossypium Stocksii* in Fig. 66. Pits in the fused walls are seen in abundance. × 300.

Fig. 10. *Thespesia populnea.* Longitudinal section of the proximal part of a bifurcate hair from the seed-coat, such as that shown in Fig. 16. Four 'pits' are to be seen within the portion of the wall between the two side lines. × 96.

Fig. 11. *Gossypium Stocksii.* Portion of a hair from the placenta within the capsule. Pits are to be seen, the dark one at the top being on the opposite wall to those showing lightly. × 300.

Fig. 12. *Thespesia macrophylla.* Stellate hair from the seed-coat, showing a reduction in the number of limbs, and with some limbs borne up upon the others. × 96.

Plate XIX.

Fig. 13. *Thespesia populnea.* A peltate scale from the petal. Side view. × 160.

Fig. 14. *Thespesia populnea.* A reduced form of peltate scale from the seed-coat. Pits can be seen between the pairs of cells. × 96.

Fig. 15. *Thespesia populnea.* A three-celled form of peltate scale from the seed-coat. × 96.

Fig. 16. *Thespesia populnea.* A two-celled form on the seed-coat, derived from a peltate scale. × 96.

Fig. 17. *Thespesia populnea.* A two-celled form on the seed-coat, derived from a peltate scale. One cell is dwindling. × 96.

Fig. 18. *Thespesia populnea.* A single cell on the seed-coat, derived from a peltate scale. × 96.

Fig. 19. *Thespesia populnea.* A peltate scale from the petal. The walls of the individual cells have not yet thickened. × 160.

Fig. 20. *Thespesia populnea.* A shield-like plate of cells from the petal. × 160.

Fig. 21. *Thespesia populnea.* A reduced form of shield-like plate from the petal. The individual cells are larger than in the previous figure. × 160.

Fig. 22. *Thespesia populnea.* Single hairs from the petal, derived phylogenetically from a peltate scale. Note how in the middle of the picture several hairs come off in a group. × 160.

Fig. 23. *Thespesia macrophylla.* Branched hair from the sterile placenta inside the capsule. × 24.

Fig. 24. *Thespesia populnea.* Peltate scale derivative with hairs fused in pairs, from the petal. × 96.

Fig. 25. *Thespesia populnea.* Peltate scale derivative with some rays squeezed into the centre, and showing as a second tier of small cells. × 96.

Fig. 26. *Thespesia macrophylla.* Stellate hair from the seed-coat. × 96.

Fig. 27. *Thespesia macrophylla.* The junction of the branched hair shown in Fig. 23. Intercommunicating pits can be seen in the wall. × 150.

Fig. 28. *Gossypium Stocksii.* Hair from a sterile placenta inside the capsule. Pits are to be seen on a knee-like swelling. × 150.

Fig. 29. *Thespesia macrophylla.* Solitary hair, derived phylogenetically from a stellate hair-group. From the seed-coat. Shear cracks can be seen. × 300.

Fig. 30. *Gossypium Stocksii.* Foot-shaped base of a hair with distal pits. From the sterile placenta within the capsule. × 150.

Fig. 31. *Gossypium Stocksii.* A similar hair to that shown in Fig. 30 but having a spur-like branch. × 150.

Fig. 32. *Gossypium Stocksii.* Bases of two hairs on the sterile placenta, showing the method of origin of the foot-shaped base by fusion of a hair with other cells. × 150.

Fig. 33. *Thespesia populnea.* The finger-like end of the longest cell in Fig. 14, showing shear cracks. × 150.

Fig. 34. *Gossypium Stocksii.* A mass of branched hairs on the sterile placenta. Some show small branches, and others are markedly bifurcate. × 24.

Fig. 35. *Thespesia macrophylla.* Two branched hairs from the sterile placenta inside the capsule. × 24.

Fig. 36. Ashmouni (Egyptian) Cotton. A branched lint hair. × 24.

Fig. 37. *Gossypium Stocksii.* A portion of the petal showing a double form of hair at the top left, and many bifurcate hairs elsewhere. × 24.

Fig. 38. *Gossypium herbaceum* var. *Goghari.* Hairs on the leaf showing one with spiral convolutions. × 24.

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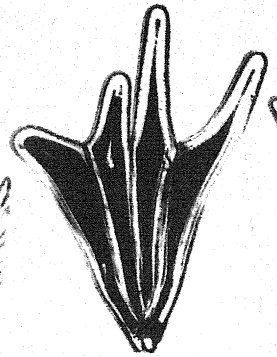
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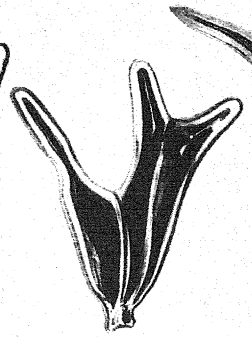
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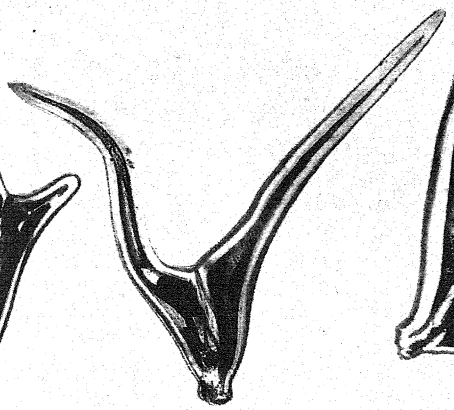
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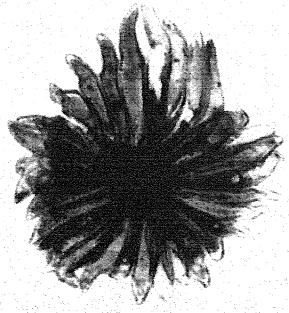
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YOUNGMAN — THESPESIA AND GOSSYPIUM.

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Chromosome Number and Relationship of some North American Species of Viola.

BY

J. CLAUSEN.

(*Genetic Laboratory of the Royal Veterinary and Agricultural College, Copenhagen*).

With sixty Figures in the Text.

INTRODUCTION.

IN a recent paper (9) the writer published the results of his study on the cytology of a number of European *Viola* species and discussed the points of general interest arising in connexion with this and preceding studies by other authors on cytology of *Viola* (13, 15).

The present paper represents a study mainly based upon collections of *Viola* material made in springtime 1928 during a stay in California. The investigations were made partly in the Genetics Laboratory of the University of California in Berkeley. The writer owes many thanks to Professor E. B. Babcock, the head of the Division of Genetics, for the kind and helpful way in which the facilities of the Laboratory were placed at his disposal, and to the International Education Board, which enabled him to spend this time as a Research Fellow of the Board.

The interval, during which meiosis takes place in these *Viola* species, is very short, and the writer had not been able to determine the chromosome number of practically all *Viola* species growing in the southern part of the California Coast Ranges, if several friends had not taken great interest in assisting him to get hold of as many species as possible, and he is very thankful to these, among whom were Professor E. B. Babcock, Mr. and Mrs. J. M. Webber, Mrs. Viola Brainerd Baird, Mrs. Dyer and Mrs. Waldron, and Mr. N. C. Fassett of Madison, Wis.

A few remarks about some technical subtleties might have some interest. Some of the fixations were made with the combination of Carnoy's fixative and S. G. Navashin's formalin-chromic-acetic acid mentioned in a paper on *Crepis* (1). Generally this proved a very good method of fixation, but it is very inconvenient when travelling; under such conditions Carnoy's fluid was used alone. In such cases an application for a shorter time than

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the ordinary 24 hours is to be recommended; the contraction is not so pronounced in Carnoy material with a time of fixation of 4 hours as with one of 24 hours.

The disadvantage of the Carnoy fixation is not only the contraction of the material, but also the fact that material so treated does not stain properly with gentian violet-iodine and sometimes not with Heidenhain's haematoxylin either; the stain disappears from the chromosomes as rapidly as from the plasma. It was found that this effect of the Carnoy fixative could be compensated for by a treatment of the slides in 1 per cent. solution of chromic acid (CrO_3) in water for one-half to two hours just before staining with gentian violet. After the chromic acid was applied, the slides were rinsed half an hour or more in water. Apparently the chromic acid acts as a mordant, as slides, which before the treatment with the chromic acid stained very poorly, after this treatment gave very clear differentiation between the deeply-stained chromosomes and the absolutely unstained plasma.

This treatment, of course, could not compensate the contraction. In all cases, therefore, the combination of Carnoy's fixative for 5–10 minutes and S. G. Navashin's fixative for about 24 hours gave the best results, and the gentian violet-iodine after this fixation gave very distinct and transparent stained chromosomes. No iodine was used before the staining with gentian violet, the chromic acid in the fixative prepares the material for the staining. The slides were stained for 5 minutes in a 1 per cent. solution of gentian violet in water, rinsed about a minute, and then treated with the iodine (1 per cent. J + 1 per cent. KJ in 70 per cent. alcohol) for about 40 seconds; next they were rapidly transferred through 50, 70, and 93 per cent. and absolute alcohol, while the differentiation proper took place in undiluted clove oil with some Orange G, in order to give the plasma a little staining.

One thing is important in applying the gentian violet: the stain must be rather freshly prepared; in some cases even two weeks' old staining fluid does not act properly. As soon as the differentiation in the clove oil does not give deeply stained chromosomes and unstained plasma, new staining fluid must be prepared.

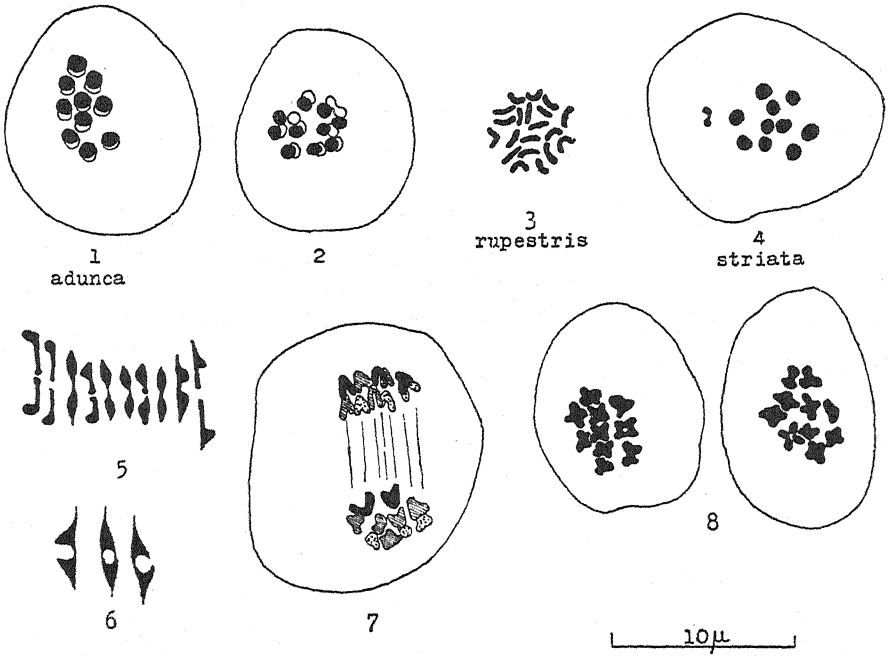
The species investigated were either collections directly from Nature, or they were types recently collected from natural habitats and implanted in gardens.

Chromosome Numbers of the Species Investigated.

The *Nominium* Section.

Viola adunca, J. E. Smith, $n = 10$. This number has been found in heterotypic metaphase of two types, one from San Bruno mountains, south

of San Francisco, collected by Mr. and Mrs. Webber (Fig. 1), and another from the top of Mt. Vision, Marin County, Cal., collected by myself (Fig. 2). The pictures displayed by the conjugated pairs of chromosomes are very



FIGS. 1–8. *Nominium* Section. When not otherwise stated, the figures are from pollen mother-cells. Magnification about 2,400 times (this is the case also with Figs 9–20).
FIGS. 1–2. *V. adunca*, heterotypic metaphases in polar view. 3. *V. rupestris*, somatic division. 4–8. *V. striata*. 4. Heterotypic metaphase in polar view. 5. The ten bivalents from one pollen mother-cell in side view showing their shape. 6. Three bivalent chromosomes in side view. 7–8. Heterotypic anaphases. 7. In side view. 8. In polar view.

clear, leaving no doubt about the actual number. In the material from Mt. Vision the chromosome pairs in heterotypic anaphase are very long and outdrawn; thus sometimes they appear smaller in polar view.

Viola rupestris, Schmidt, var. *arenaria*, (D. C.) G. Beck., $2n = 20$ (Fig. 3). The plant investigated was grown from seed collected on natural habitat in Caucasia and sent by Dr. G. Voronov, once of Tiflis. The count is from a very good and convincing metaphase in somatic tissue in the floral region.

V. striata, Ait., $n = 10$ (Figs. 4–8). This species was obtained from a typical population in the Botanic Gardens of the University of Michigan, Ann Arbor. Here also the number was unquestionably 10. Although fixed with Carnoy for four hours, the details of the meiosis were comparatively clear. The shape of the metaphasic chromosomes in this species was different from that seen in all violets hitherto investigated. In diaphase (= diakinesis) the gemini are not dumb-bell shaped as in other violets but constitute crosses, rings, parallel rods with connexions, &c., much like the

Crepis gemini studied by the present author (1), and which showed clear cases of chiasmas and cytological crossing over between chromatids of homologous chromosomes. In *V. striata* the chromosomes unfortunately are too small for a study of the details of meiosis, and the Carnoy fixation has not preserved this phase well enough for such a study of details.

In heterotypic meta-anaphase, as seen in side view (Figs. 5 and 6), the chromosome pairs have strikingly different shapes, sometimes J-like, sometimes rodlike, sometimes ring-shaped. In some cases the disjunction of the conjugated chromosomes apparently is difficult; they are much outdrawn, and knots left at the place of conjugation indicate how close this has been. In late anaphase the chromosomes become V-shaped, and as they furthermore cleave early for the homotypic division they generally appear four-branched when seen in polar view (Figs. 7 and 8). In a few pollen mother-cells an extra diminutive chromosome-like body was seen, sometimes dividing (Fig. 4).

Finally, it can be added that the European *V. uliginosa* Bess. has $n = 10$, counted in a number of very clear heterotypic metaphases.

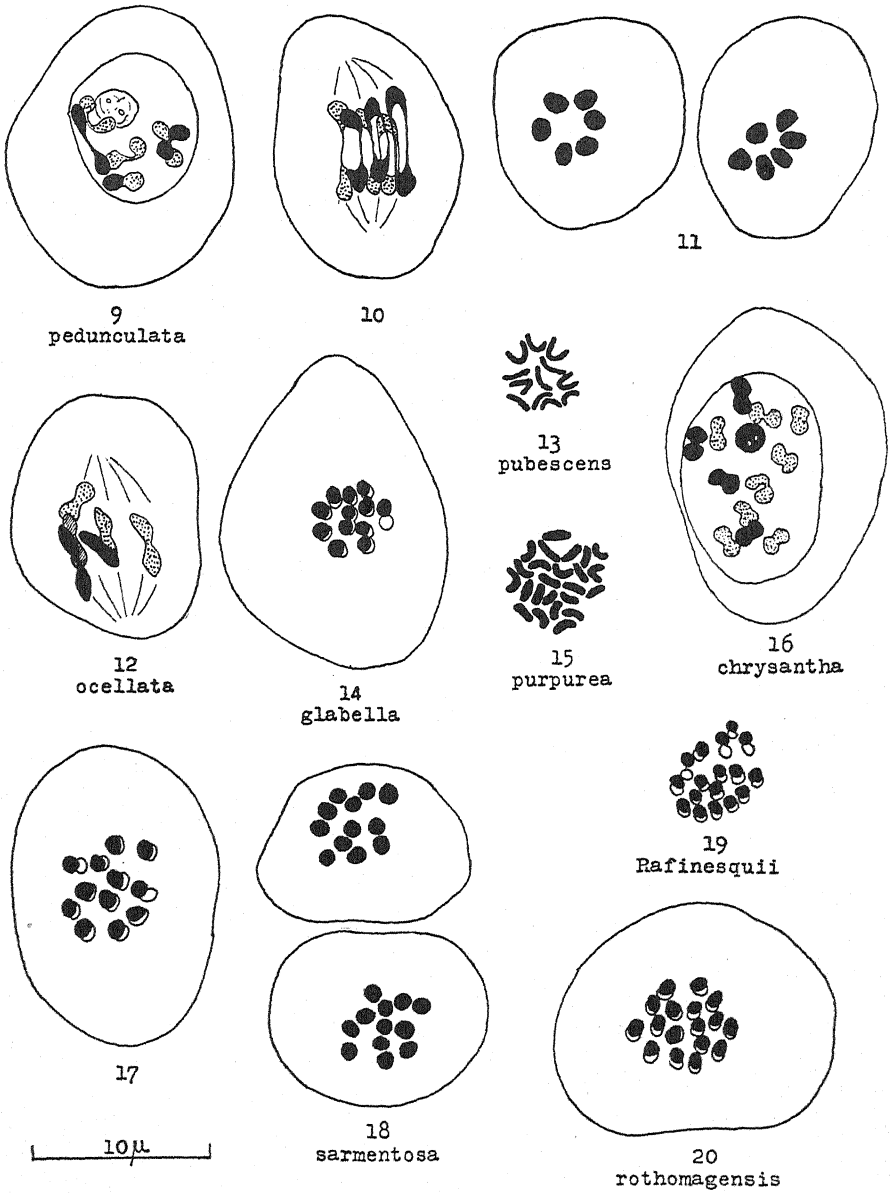
The *Chamaemelanium* Section.

V. pedunculata, Torr. et Gray, $n = 6$ (Figs. 9–11). This interesting *Chamaemelanium* violet, the Californian so-called 'yellow pansy', is a type growing on hillsides, its digitate root-stocks anchored deep in the crevices of the rocks. During the rainy season it sends many long runners up from these root-stocks, forming dense mats crowded with big, intensely yellow flowers in the shape of those of a large-flowered *V. lutea*. The shape of the flower superficially recalls a *Melanium* violet, but the characters of the vegetative system, and also those of the style, place it among the *Chamaemelanium* violets. Although the species has a rather wide range of distribution in California, there is not much variation in it, perhaps owing to its low chromosome number.

The investigated type was collected by Mr. and Mrs. Webber on San Bruno mountains, San Francisco peninsula. The chromosomes are rather large, somewhat V-shaped as seen in side view (Fig. 10), and the number of six chromosomes is seen clearly in the diaphase (Fig. 9), heterotypic metaphase (Figs. 10 and 11), as in homotypic metaphase.

V. ocellata, Torr. et Gray, $n = 6$ (Fig. 12). The count was made upon a wild type plant transplanted to a garden. The number was counted in late diaphase and early heterotypic metaphase.

V. pubescens, Ait., $2n = 12$ (Fig. 13). Typical *V. pubescens* were collected in a small wood near Madison, Wis. It was too late for meiosis. All the flower-buds would give cleistogamic flowers, but a number of somatic divisions in young eggs showed unquestionably twelve chromosomes.



FIGS. 9–18. *Chamaemelanium* Section and 19–20 *Melanium* Section.

FIGS. 9–11. *V. pedunculata.* 9. Diaphase. 10–11. Heterotypic anaphases. 10. In side view. 11. In polar view. 12. *V. ocellata*, early heterotypic metaphase in side view. 13. *V. pubescens*, somatic division. 14. *V. glabella*, heterotypic metaphase in polar view. 15. *V. purpurea*, somatic division. 16–17. *V. chrysantha.* 16. Diaphase. 17. Heterotypic metaphase in polar view. 18. *V. sarmentosa*, heterotypic anaphase in polar view. 19. *V. Rafinesquii*, heterotypic metaphase in polar view. 20. *V. rothomagensis*, heterotypic metaphase in polar view.

V. glabella, Nutt., $n = 12$ (Fig. 14). The material was collected from a wild type, transplanted to a garden. Also material from Redwood forest in Santa Cruz mountains, north of Monterey Bay, was fixed, but proved too old. Meantime, the type from the garden and the wild growing types were quite identical in appearance. In two fixations, one in Carnoy and 45 per cent. acetic acid, and another in Carnoy-Navashin, the chromosomes appeared somewhat conglomerated, perhaps not owing to any fault in fixation, but to the fact that it was too late in the flowering season, the pollen mother-cells being somewhat degenerated. Notwithstanding, material of the last fixation fairly certainly showed twelve bivalents in heterotypic metaphase.

V. purpurea, Kell., $2n = 24$ (?), (Fig. 15). This species was collected by Mrs. Brainerd Baird on Mt. Veedar in Napa County, Cal. The count was made on somatic divisions in the ovary. The nuclear plate in Fig. 15 has two places where the count is made with a little reservation, but it cannot bring the number under 23 and not above 25, and the number of 24 is the most likely one, if judged from the appearance of the chromosomes in question.

V. chrysantha, Hook., $n = 12$ (Figs 16–17). This interesting species was collected at Tehachapi pass, between Mohave and Bakersfield, Kern County, altitude about 1330 metres. The deeply yellow colour of the flower, and especially the finely pinnatifid leaves somewhat in the shape of a *Ranunculus*, give it an appearance strange to a violet. It grew in abundance in the place. The fixation was with Carnoy, and it would not stain before after-application of chromic acid. But after this treatment it stained very beautifully and gave very clear counts both in diaphase and in heterotypic metaphase as well.

V. sarmentosa, Dougl., $n = 12$ (Fig. 18). This characteristic evergreen violet trails with its prostrate, stolon-like stems the ground in the Redwood forests, very much alike *Linnaea borealis*. The chromosomes were often somewhat conglomerated; still such a pollen mother-cell as that in Fig. 18 clearly showed $n = 12$ in two corresponding heterotypic anaphase plates. This was from a garden transplant; but material from Redwood forest in Santa Cruz mountains also showed $n = 12$ in homotypic metaphase.

The *Melanium* Section.

Viola Rafinesquii, Greene, $n = 17$ (Fig. 19). Strangely enough, the *Melanium* section, which is a European section with some few outrunners in the mountains of Western Asia and Northern Africa, has one representative, *V. Rafinesquii*, in the eastern part of North America. Morphologically, it would be classified among the *Tricolores* in the circumscription these were given in a former paper (9), and it might have resemblance to some of the larger flowered *Kitaibeliana* types. The plants were grown

in Denmark from seeds benevolently sent me by Dr. A. Gershoy of Burlington, Vermont. Meiosis found in pollen mother-cells of some few chasmogamic flowers showed regular divisions and 17 bivalent, rather small chromosomes (Fig. 19).

The most western type of *V. Kitaibeliana* in Europe, *V. nana* D. C., has $2n$ = about 48 (not more, and no less than 46). Heterotypic divisions are not so clear as the somatic ones owing to irregularities, caused, likely, by too many possibilities for conjugation in this apparently octoploid type. I owe thanks to Dr. E. Drabble, who kindly sent me the seeds of this very interesting type collected at St. Queen's Bay on the small island of Jersey in the English Channel.

V. rothomagensis, Desf. (Fig. 20). The chromosome number of this species was published by the present writer in a former paper (9, p. 694, and Fig. 68), but the material was somewhat scarce and the staining dark; still, it showed clearly $n = 17$ in the homotypic metaphase. Meantime Gershoy (10) found $2n = 36$, and therefore some new material of later generations was fixed with Carnoy-Navashin and stained in gentian violet. In this was an abundance of divisions of pollen mother-cells, diaphases, heterotypic metaphases and anaphases and homotypic anaphases, very clear and beautifully stained, and all showing $n = 17$. Fig. 20 shows just one of these very regular heterotypic metaphases.

Recently Gershoy (10) published a preliminary paper on chromosome numbers of 59 *Viola* species, mainly North American. The diploid number is given, indicating that the counts have been made on somatic tissue, but the paper is not accompanied by any figures. Of the 21 species which both he and the present writer investigated, there is accordance only in 9 species, while in 12 species the countings differ. It is easily understood that there must be a certain margin of uncertainty in the countings made on somatic plates. Somatic *Viola* chromosomes are difficult to count with certainty, due to the fact that they very often adhere end to end, in such a way that it is impossible to settle if, for instance, an apparently single chromosome really is one bent in the shape of a V, or two chromosomes placed end to end. The present writer, therefore, dare not count with certainty somatic chromosome numbers in *Viola* larger than about 20, while in pollen mother-cells the chromosomes are much more distinctly and easily counted. In a table on page 748 are given the cases of disagreement between the countings of Gershoy and those of the present writer.

In most cases Gershoy's counts are very close to the present ones. He counts for instance $2n = 18$ where the present count is $n = 10$ and $2n = 36$ as compared with $n = 17$, &c. The present writer maintains his earlier counts. Most often the chromosome plates were so clear that a miscounting was impossible, and it must be remembered how much more definite the numbers can be determined during meiosis than in somatic divisions.

The Cases of Discrepancy in the Chromosome Numbers of Viola:

	Gershoy (10).	Clausen (9).
striata, adunca, odorata	$2n = 18$	$n = 10$
silvestris	$2n = 42$	$n = 10$
elatior	$2n = 42$	$n = 20$
papilionacea	$2n = 54$	$n = 26$
sarmentosa	$2n = 42$	$n = 12$
purpurea	$2n = 30$	$2n = 24$ (?)
tricolor, 3 varieties	$2n = 24$	$n = 13$
arvensis	$2n = 36$	$n = 17$
rothomagensis, Rafinesquii	$2n = 36$	$n = 17$
cornuta	$2n = 42$	$n = 11$

A renewed investigation was made on *V. papilionacea*, formerly (9, p. 186), published as *V. cucullata*. Gershoy's count here was $2n = 54$, as compared with $n = 26$ of the present writer. The number is so large, and the chromosomes relatively so small, that it called for a re-inspection. But all good chromosome plates showed $n = 26$, leaving no doubt as to the correct number in this case. Whether this is true for the other 19 species of *Boreali-Americanae* W. Becker (including *Pedatifidae*, W. Becker), in which Gershoy counted $2n = 54$, cannot be told, but it seems likely that the species of this isolated block of difficult distinguishable types all have the same, or almost the same, chromosome number and a number different from that of most other *Viola* species.

V. rothomagensis was mentioned above. The new preparations leave no doubt about the fact that this species has not $n = 18$ but $n = 17$ chromosomes (Fig. 20). As for *V. tricolor* and *arvensis* the present writer has investigated many varieties, and always found $n = 13$ and $n = 17$ respectively; the numerous hybrids of these species investigated also indicated these numbers as the correct ones. Irrelevant types, only, had different numbers and univalents, but in such case showed numbers between those of the two species. *Tricolor* with $n = 12$ likely would not be viable.

There is not much probability that the haploid and the somatic chromosome number of the species should not correspond. A species as *V. rupestris*, which by its relationship ought to have $2n = 18$, if Gershoy's numbers are the correct ones, very clearly shows $2n = 20$. In *purpurea* both Gershoy's and the present countings are in somatic cells. Although the present count is not quite certain, it is by no means 30, but between 23 and 25; most probably 24.

Some of the discrepancies, however, are so large that they call for some more explanation. In *V. silvestris*, where the two counts are $2n = 42$ and $n = 10$ respectively, the type investigated by Gershoy probably has been *V. Riviniana* ($n = 20$). The two species resemble one another very much, and are confounded in most gardens. Likewise Gershoy's *V. cornuta* with $2n = 42$ probably is '*V. cornuta hybrida*' (= *V. Williamsii*, Wittr.), a

garden type of hybrid origin often given as *V. cornuta*. It has n = about 24 of which there are often various univalents (9, p. 696, Fig. 77). The count $2n = 42$ for *V. sarmentosa* as compared with $n = 12$ is more difficult to account for, but Gershoy's type might be a tetraploid one ($2n = 48$?).

In analogy with this we might be justified in suspecting *V. conspersa, labradorica,* and *rostrata* having not 2n = 18 but $n = 10$, and *V. incognita* to have not $2n = 42$ but $n = 24$. Likewise, due to its relationship, it should be expected that *V. praemorsa* should not have $2n = 30$ but, similar to *purpurea*, $2n = 24$ ($n = 12$). What the right number of *V. Howellii* (Gershoy: $2n = 42$) should be, cannot be foretold, as its systematic position is not quite clear. If it belongs in group with *V. adunca*, it might in this group be the only American parallel to the European tetraploid *V. Riviniana* ($n = 20$).

It must be mentioned that in the cases of *V. ocellata, pubescens*, and *glabella* the present counts verify Gershoy's previous ones. It is remarkable that Miyaji (15) found $n = 6$ for Japanese *glabella*, while Gershoy and I both find $n = 12$ for the American type, which accordingly must be regarded as a tetraploid as compared with the Japanese type.

In other respects Gershoy's paper is of great interest. The list of successful hybrids, some of which are very widespread, as the hybrid *V. palustris* ($n = 24$) × *V. biflora* ($n = 6$), is remarkable. The detailed report about the behaviour of some of these hybrids must be awaited with much interest. Likewise his study of pollen germination on alien stigma shows that one of the reasons why crossings between the *Melanium* and the *Nominium* sections are unsuccessful is the remarkable retardation in the growth of the pollen tube on media so different.

Discussion.

Viola adunca, and its Systematic Position.

By many American botanists *V. adunca* has been treated as a near relative, sometimes a variety, of *V. canina* L. As Becker already (3, p. 33) has shown, this is wrong. The life-cycle of *adunca* is very different from that of *canina*. It belongs to the *Rosulantes*; the main stem ends in a rosette, while *canina* has no rosette. In fact, all the American violets of the *Rostellatae* group have this rosette; probably this is the reason why the American botanists generally have neglected this significant morphological difference. The *Arosulatae*, to which *canina* belongs, is an exclusively Eurasian group. Furthermore, the chromosome number of *adunca* has nothing to do with that of *canina*; the only *canina* type investigated has $n = 36$, while *adunca* had $n = 10$.

But *V. adunca* has much more resemblance to *V. rupestris* Schmidt of the *Rosulantes* group. Both species belong to the open land, while their

nearest European type, *V. silvestris*, is a decided woodland type. The *adunca* type investigated, which grew on the exposed top of Mt. Vision with the ocean wind blowing across it, really was very similar to some Eurasian types of the collective species *V. rupestris*; it had cordate, somewhat rigid, puberulent leaves, and a condensed, tufted-growth form; still, it had the hooked spur and a style a little different from that of *rupestris* (Figs. 50, 51). Nevertheless, if it had grown on the other side of the Pacific Ocean it would have passed as a good *V. rupestris*. Some of the Eastern American types, which pass under the name of *V. adunca*, it must be admitted, are a little more different from *rupestris*, but one really would expect some variation within a species with such a wide range of distribution. So, notwithstanding Brainerd's retaining of *V. adunca* as a definite species (7), the present writer proposes to follow W. Becker (3, p. 62) in treating *adunca* as a subspecies of *rupestris*, in view of the fact that the western *adunca* types form the connecting link with *rupestris*, and also because the chromosome numbers do not give any reason for a separation of them.

Taxonomic Considerations.

Since my last paper on chromosome number and relationship (9) appeared so many new chromosome numbers have been added that it must be time for a reconsideration and a re-grouping of some of the sections. The relationship displayed in the chromosome numbers really bring to one's mind some morphological characters, which largely follow the chromosome series, and which in connexion with these might be used in future classification of the violets.

Although such a reclassification at the present juncture must be somewhat tentative, owing to the fact that the chromosome numbers of many species are still unknown, some new light might be thrown on the question of the origin and the home of the violets of the northern hemisphere.

In a table below, the chromosome numbers now known are briefly listed as they appear after the investigations of Miyaji (15), Heilborn (13), Gershoy (10), and the present writer (9). The species are grouped as I would prefer to group them in view of resemblance in macro-morphological and cytological characters. Some group names have been adopted from one of W. Becker's earlier papers (2), as they seem more adequate for the new classification than some of his later ones (4):

Chromosome Numbers of Viola.

Subsect. NOMINIUM-ROSTELLATAE, Boiss.

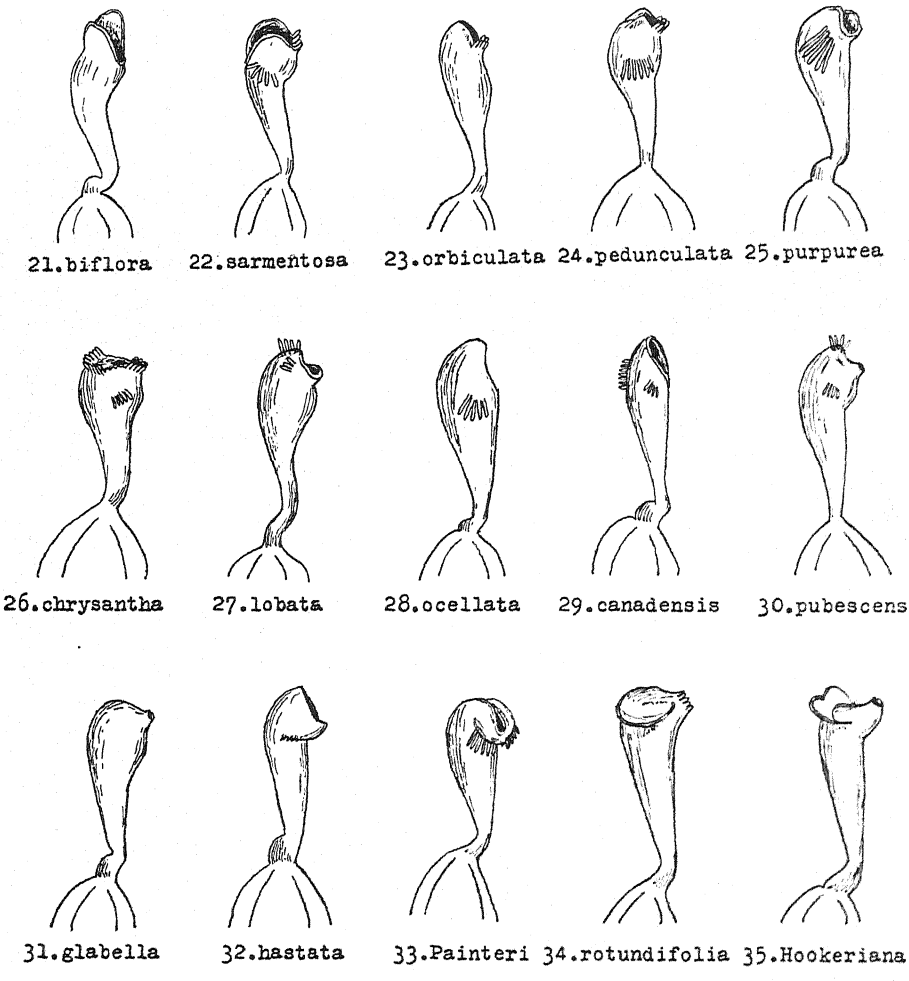
	$n =$
I. Scapigerae, W. Beckr.	
odorata, *hirta*, *nipponica*	10
(*sepincola*. Jord. is likely tetraploid).	

	n =
II. Rosulantes, Borb.	
mirabilis, silvestris. grypoceras, rupestris, adunca, striata	10
(*labradorica, conspersa, rostrata*	likely 10)
Riviniana, neglecta	20
III. Arosulatae, Borb.	
stagnina (= *persicifolia*, Roth), *uliginosa*	10
elatior	20
canina	36
IV. Bilobatae, W. Beckr.	
verecunda	10
Subsect. NOMINIUM-PLAGIOSTIGMA, Godr.	
V. Stolonosae, Kupffer.	
epipsila, pallens, primulifolia, lanceolata, renifolia	12
palustris, blanda (and likely *incognita*)	24
VI. Adnatae, W. Beckr.	
Keiskei, phalacrocarpa, Selkirkii	12
japonica, mandschurica, pinnata	24
Patrini	36
VII. Diffusae, W. Beckr.	
diffusa	13
VIII. Boreali-Americanae, W. Beckr.	
papilionacea	26
pedata, palmata, cucullata, latiuscula, missouriensis, Lovelliana, triloba, sororia, hirsutula, septentrionalis, affinis, Langloisii, nephrophylla, villosa, fimbriatula, sagittata, emarginata, Brittoniana, septemloba	(possibly 26) 27
Sect. CHAMAEMELANIUM.	
I. Biflorae ined.	
biflora	6
sarmentosa	12
II. Nuttallianae, W. Beckr.	
pedunculata	6
purpurea	12
III. Chrysanthae W. Beckr.	
chrysantha	12
IV. Rotundifoliae ined.	
rotundifolia	6
V. Erectae-Canadenses, W. Beckr.	
ocellata	6
rugulosa, canadensis	12
VI. Erectae-Nudicaules, W. Beckr.	
lobata, pubescens, eriocarpo, Japanese *glabella*	6
American *glabella*	12
Sect. MELANIUM.	
I. Calcaratae, W. Beckr.	
cenisia, declinata, elegantula	10
cornuta, orthoceras, Orphanidis	11
calcarata, Zoysii	20
II. Tricolores, W. Beckr.	
Kitaibeliana	7, 8, abt. 12, 18, abt. 24
tricolor, alpestris	13
arvensis, rothomagensis, Rafinesquii	17
lutea	24
Munbyana var. *Battandierii*	[1] abt. 30

[1] This old count may not be quite certain, as it was made on some few homotypic metaphase plates. Some crossings made with this type as a parent indicate that gametes with less than thirty chromosomes can be formed. The type is now contaminated by spontaneous crossings.

Sect. LEPTIDIUM.

	n =
Humboldtii	27
Hybanthus parviflorus	12

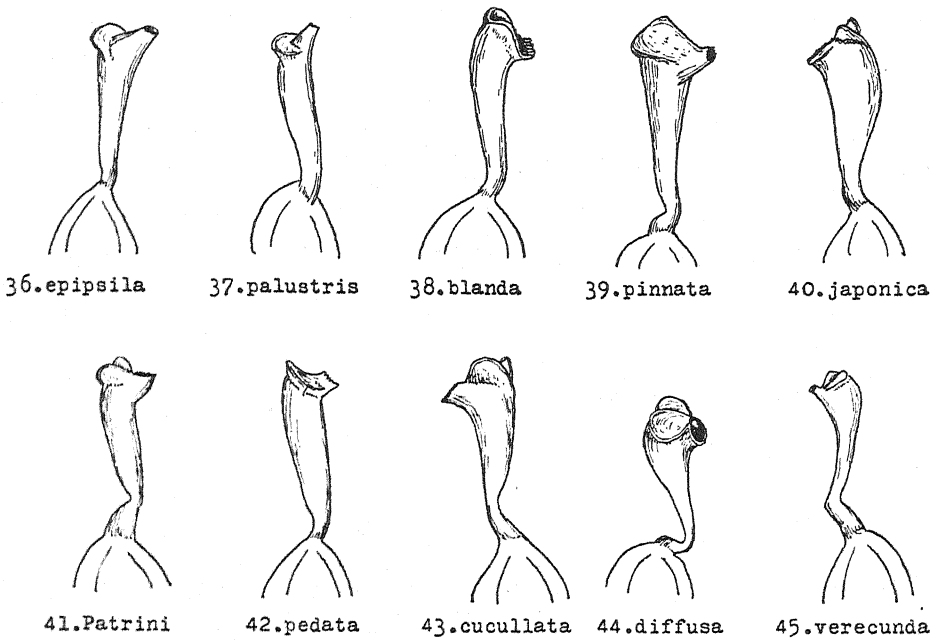


FIGS. 21–35. Style-shapes of species belonging to the *Chamaemelanium* Section. Magnification 12 to 20 times.

An essential point in previous classifications of the violets, as well in Gingins' (11) as in Becker's (4), has been the shape of the style. In order to estimate the significance of this character, the writer made a study of style-shape in most of the violets with known chromosome numbers. In Figs. 21–57 are shown the shape of the style of a number of these species.

The most uniform shape of style is found in the subsection *Rostellatae*

of the *Nominium* section (Figs. 46–55). The stigma is placed on the end of a rostellum, which ends the pistil; this rostellum is more or less curved forwards. The flower colour of these violets is purplish blue or bluish white, never yellow. It happens that the group is practically identical with the group of 10- or 20-chromosome *Nominium* violets.



FIGS. 36–44. Style-shapes of species belonging to the *Plagiostigma* Subsection.

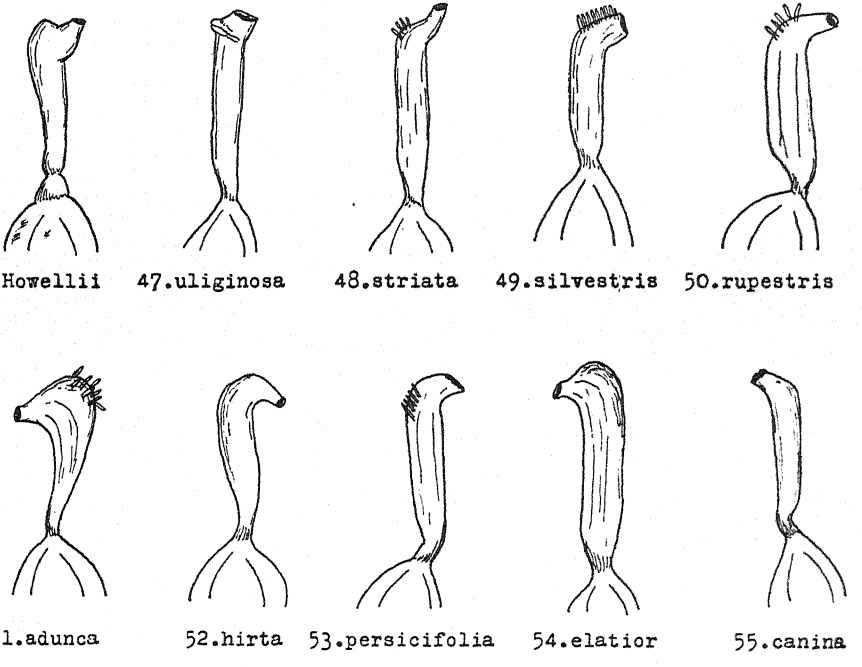
FIG. 45. Style of *V. verecunda*, most naturally classified with the *Rostellatae*.

The remaining *Nominium* violets belong to a 12-series, having as aberrations 13- and 26-chromosome groups. The styles of all these are widened out at top, and most often helmet-like, due to more or less protruding edges (Figs. 36–43). In most cases the stigma is situate on front of a little rostellum. The flower colour is the same as in the preceding group, purplish-blue or bluish-white, not yellow. One group has stolons (*Stolonosae*), the others are totally stemless (*Adnatae* + *Boreali-Americanae*); none have erect stems. In this circumscription the group will carry the name *Plagiostigma*, Godr.

A difficulty is caused by *V. verecunda* Gray of the group *Bilobatae*. The style-shape is much the same as in *Plagiostigma* (Fig. 45), but the growth-form and the chromosome number, which are the same as in *V. silvestris*, place it more naturally in connexion with the *Rostellatae* as an interesting transition type to the *Plagiostigma* subsection.

The former *Chamaemelanium* section contains no blue or bluish violets,

all are yellow or whitish, and they have either 6 or 12 chromosomes. All these yellow violets attain their most numerous development of types along the North American Pacific coast, branching out to the Eastern States and to East Asia. In all other characters than the flower colour and the



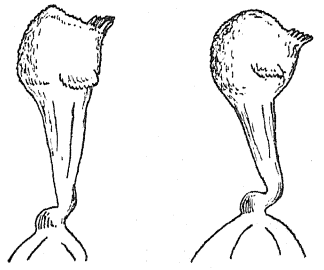
FIGS. 46–55. Style-shapes of species belonging to the subsection *Rostellatae*

chromosome number these violets show a great variation, even the style is exceedingly varied, although most of them have a capitate one (Figs. 24–33).

But in the same area also some other yellow violets exist, which Becker (4, p. 369) referred to the *Nominium* section (*Orbiculares* and *Mexicanae*). In view of the fact that the yellow flower colour is so strong an indication for *Chamaemelanium*, and also because they live within its natural range of distribution, the writer proposes to remove them to this section. In *Orbiculares* Becker placed *rotundifolia* ($n = 6$, Gershoy), *sarmentosa* ($n = 12$), and *V. orbiculata*. Of these, *sarmentosa* and *orbiculata* doubtless are near related, both as regards vegetative characters and as regards style-shape (Figs. 22, 23), while *rotundifolia* ought to be placed in a very different group. Its style-character (Fig. 34) resembles *V. Hookerianae* of the *Mexicanae* group (Fig. 35), and both of them form transition types to *Plagiostigma*. The low chromosome number of 6 in *V. rotundifolia* places it more naturally among *Chamaemelanium* than in *Plagiostigma*.

One group more can naturally be included in the *Chamaemelanium*

section. Gingins (11) placed between the *Nominium* and *Chamaemelanium* sections the *Dischidium* section, the contents of which have been diminished ever since that time, and which now mainly consists of one circumpolar species, *V. biflora* L. This species is yellow-flowered like *Chamaemelanium*, and has a somewhat club-shaped pistil, with a rather deeply-cleft stigma at top (Fig. 21); the chromosome number is 6, finally. The writer cannot see any real reason for separating it from the *Chamaemelanium* section. Morphologically, *V. sarmentosa* ought to be its nearest relative. The style of *sarmentosa* is cleft, although not as deeply as in *biflora* (Fig. 22), and the growth-type, the shape of the leaves and of the flowers are much the same in the two species, except that *sarmentosa* is evergreen. Thus *V. sarmentosa* would be the tetraploid species corresponding to the diploid *biflora*. On the other hand, the style-shape of *sarmentosa* is not very different from that of *V. pedunculata* (Fig. 24), a true *Chamaemelanium* species. In this way, as regards the style-shape, these three species—*biflora*, *sarmentosa* and *pedunculata*—form a handsome series of transitions, although they formerly, just based upon the style-type, have been placed in three different sections. The other characters place *biflora* and *sarmentosa* together with *orbiculata* (Fig. 23) in quite another group of the section than that to which *V. pedunculata* belongs.



56.cornuta 57.Rafinesquii

FIGS. 56–7. Styles of two species belonging to the *Melanium* section.

The violets of the *Chamaemelanium* section, as regards chromosome number, appear more primitive than those of the *Plagiostigma* subsection, as they have either 6 or 12 chromosomes while *Plagiostigma* has formed a real 12-series.

The *Melanium* section, finally, is more isolated and separated from the others by several characters, specially by the upturned side petals (in the other sections downturned) and by the big divided and leaf-like stipules. The style is still more capitate than in *Chamaemelanium* with a big stigma in its hollow and a labellum under the entrance to the stigma (Figs. 56, 57). The flowers are either yellow, purplish blue or whitish. As regards chromosome number it is the most perplexing section, although it shows indications of a 6-series and a 10-series with many aberrations.

Phylogenetic Considerations.

If similar points of view to those Vavilov (17) applied to the grasses can be applied also to the *Violaceae*, then their home should be the tropic belt, and especially tropical South America. The greatest number of genera,

as *Rinorea*, *Paypayrola*, *Amphirrhox*, *Gloespermum*, *Anchietia*, *Coronostylis*, *Isodendrion*, *Allexis*, *Agathea*, and *Melicytus*, belong to the tropics, *Hymenanthera* to the subtropics, and *Hybanthus* to both tropics and subtropics. *Viola* is the only genus in the family which has its main area in the temperate belt, while in the tropics it inhabits the mountainous areas. This probably is a consequence of the fact that while all the other genera mainly consist of phanerophytes (the life type specially adapted for the tropical climate) the genus *Viola*, mainly contains hemicryptophytes, the most common life type of the temperate regions (Raunkiaer, 16).

Also, from a morphological point of view, the tropic genera of the *Violaceae* are the more primitive. The flowers of these genera are mainly actinomorphic or faintly zygomorphic, more near to the common flower type in other families, while the genus *Viola* consists of only zygomorphic types, a flower type *sui generis*.

Within the genus *Viola* we have the same play of variation as between different genera of the family; Southern America has the greatest number of types more nearly related to the common stock of *Violaceae*—small shrubs and chamaephytes adapted to the mountain districts.

In the writer's opinion we find the prototypes of the violets of the temperate region in Mexico and along the Pacific coast of North America. We find these types in the *Chamaemelanium* section, which constitutes the connecting link between the South American types on one side and the *Rostellatae*, *Plagiostigma*, and *Melanium* violets of the northern temperate zone on the other.

The extended variation within the *Chamaemelanium* section has been referred to previously, both as regards the shape of style and stigma and also as regards growth form. The stemless *Plagiostigma* violets show resemblance to the *chrysantha* group. The style of *chrysantha* (Fig. 26) is not so much different from a number of *Plagiostigma* styles (Figs. 39–43). The pinnatifid and partite leaves in *chrysantha*, *Sheltonii*, *Beckwithii*, *Hallii*, and *trinervata* of the *crysantha* group recall the partite leaves in *pedata*, *pinnata*, *dactyloides*, &c., of the *Plagiostigma* subsection.

V. pubescens and *glabella* have strong resemblances to *Rosulantes* and *Arosulatae* of the *Rostellatae*; the style even has a little rostellum (Figs. 30, 31); in growth form also *V. ocellata* and *canadensis* are similar to members of these groups, particularly the *Rosulantes*.

Again, types of *V. pedunculata* and *purpurea* remind so strikingly of *Melanium* that one sometimes doubts whether *pedunculata* belongs to *Melanium* or to *Chamaemelanium*. The leaf shape is repeated on a smaller scale in *V. paradoxa* Lowe from Madeira (*Melanium* section), while the flowers recall large-flowered, intense yellow *Viola lutea*. The styles of *purpurea* (Fig. 25), *pedunculata* (Fig. 24), and particularly *lobata* (Fig. 27) show strong resemblances to the *Melanium* section.

Perhaps the most primitive group of the *Chamaemelanium* section is the *biflora-sarmentosa* group, a line which has not been carried further. *V. rotundifolia* also stands a little isolated.

If we consider the development of the style only, the cleft one must be looked upon as the most primitive type (Figs. 21–3); from this there is not far to the flattened and disc-like one (Fig. 26). In *V. purpurea* the stigma has become covered but has a big entrance on one side (Fig. 25). When this opening becomes smaller and protruded on a small rostellum, we have the *glabella* type (Fig. 31). Next, the head of the pistil becomes less pronounced, the rostellum with the opening of the stigma more protruded, and the *Rostellatae* type has been formed (Figs. 50, 51, 54). For such a development perhaps the accumulation of small genic differences has been responsible. The manifold variation of style-types found in the *Chamaemelanium* section, and also in other sections, really suggests a large number of genes co-operating in determining the style-shapes.

The fact that the *Rostellatae* subsection has become fairly uniform as regards style-shape does not necessarily indicate that this character is in causal relation to the 10-series of chromosomes. It only indicates that these violets have possibly become homozygotic in a certain number of genes determining the style-shape (also in genes determining the flower colour), while the fact that they belong to the same 10-series indicates that they have formed a distinct line of development.

Although the writer does not believe much in the value of speculation about the phylogenetic lines along which the development of a genus has taken place, he thinks that in the violets by the use of both morphological, cytological, genetical, and geographical principles some new light might be thrown on the origin and the development of the violets of the northern hemisphere. In Fig. 58 he has tried to present his ideas in a phylogenetic chart.

With our present knowledge we can judge six to be the basic and primitive chromosome number of the violets. Six is the lowest number found, and the 6-series is found among the most primitive ones hitherto investigated. Furthermore, the number 12 is also determined for a species of the primitive genus *Hybanthus* (Heilborn, 13). The *Chamaemelanium* section, therefore, of which fourteen species have been found to have either 6 or 12 chromosomes, has been placed central with connexions to the other sections and subsections. Also in other respects it appears to occupy a central and primitive position. It must be assumed to have its roots somewhere in South America, but to have come up through the narrow land connexion of Central America and developed along the Pacific coast a series of types. Only one of these, perhaps the most primitive of them all, namely *biflora*, became circumpolar, but some few spread to Eastern North America, and some others to East Asia (Fig. 60). Along different parallel

lines it developed tetraploidy, as in the cases of *purpurea*, *chrysantha*, *canadensis*, and *glabella*, and perhaps both tetraploidy and octoploidy in *sarmentosa*.

The group most nearly related to it doubtless is the *Plagiostigma* subsection of *Nominium*. Really, if some of the *Plagiostigma* violets were

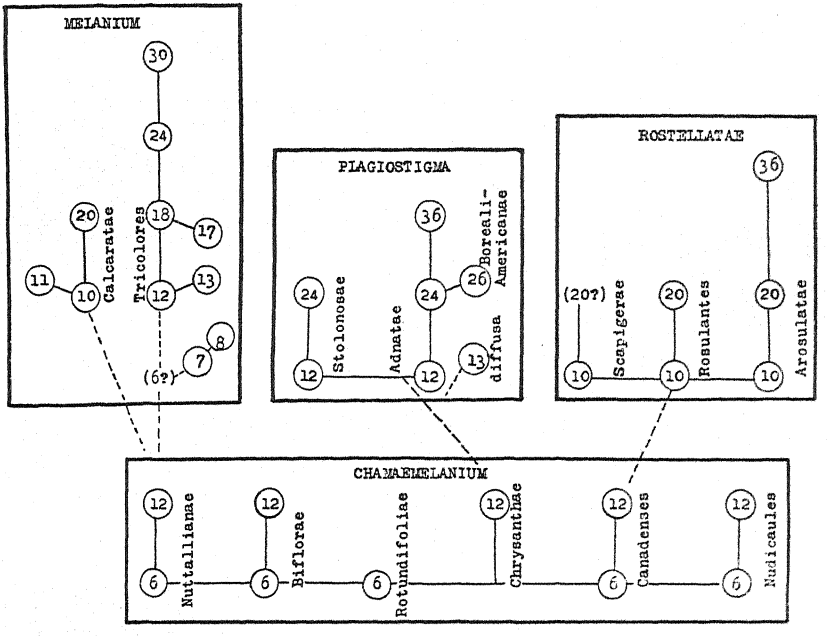


FIG. 58. A tentative phylogenetic chart of the four main groups in which the violets of the northern hemisphere are classified.

yellow-flowered they would be difficult to distinguish from *Chamaemelanium*, except by chromosome number in some cases. Also the number of successful hybrids between *Plagiostigma* and *Chamaemelanium*, according to Gershoy (10), indicates the close affinity (Fig. 59). A 12-series of chromosome numbers has been developed in *Plagiostigma*, so that one is tempted to assume 12-chromosome types as the starting-point for the subsection. It seems to have started along two main lines: the *Stolonosae*, which became circumpolar, and the *Adnatae*, which had their greatest development in Eastern Asia, and here created also a 36-chromosome type. By an aberration in chromosome number the *Boreali-Americanae* were isolated from their nearest relatives, *Adnatae*, but they developed in Eastern North America numerous types, apparently without any change in chromosome number, by Mendelian combination only. Whether the *Boreali-Americanae* consists of only one Linnean species with many ecotypes, or it really forms a number of nearly related and intercrossing species, is a matter of question, about which different opinions can be maintained. But Brainerd's beautiful

investigations on hybrids in this critical group (5, 6, 8) indicate how some of the species might be differentiated out after crossings of other species. *Diffusa* in Asia might be a similar deviation from 12-chromosomed *Adnatae*.

The numerous violets of the *Rostellatae* subsection may have their nearest relatives among the *Canadenses* of the *Chamaemelanium* section.

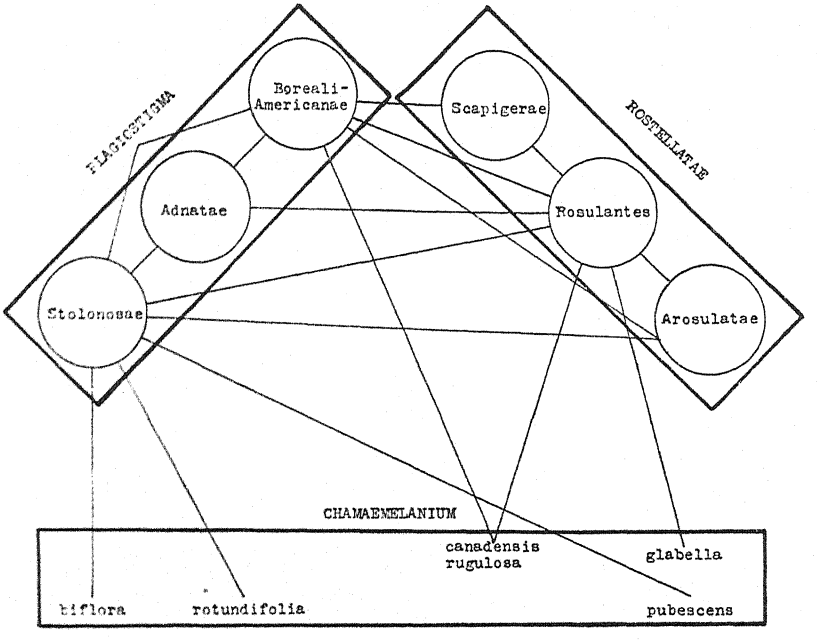


FIG. 59. The known cases of hybridism between different groups of *Chamaemelanium*, *Plagiostigma*, and *Rostellatae*, based upon records on hybrids occurring in Nature, and upon Gershoy's list of successful crossings (10). Lines between groups indicate hybrids.

Gershoy (10) made several successful crossings between this group and the *Rosulantes* of *Rostellatae* (Fig. 59). The *Rostellatae* developed three main groups: two stemmed ones, the *Rosulantes* with a circumpolar distribution (Fig. 60), and the *Arosulatae*, which are Eurasian only, and finally also a stemless group, the *Scapigerae*, of which the most numerous types are Mediterranean or West Asiatic; some of the *Scapigerae* still have creeping stolons, but none have erect stems as in the two other main groups. How this subsection of violets jumped from a 6-series to a 10-series we cannot tell; perhaps by some crossing, perhaps in another way; in a previous paper (9, pp. 707–10) the writer has discussed these problems. The possibility, however, cannot be denied that they may have an origin different from that of the 6-series violets, in a manner that we here had a case of convergent evolution; but the fact that many of them can be crossed with violets of the 6- and 12-chromosome series (Fig. 59) really strongly supports

the view that they originated from a common, not too remote, source. Still, it seems as if they are able to reproduce secondary 6-series violets as in the case of the investigated *canina* type with $n = 36$; it would not be surprising to find other types of this variable species with other chromosome numbers.

Each of the three groups of the *Rostellatae* have developed tetraploid types. In the *Scapigerae* group the tetraploid type is represented by *V. sepincola* Jord. The chromosome number has not been determined exactly, as sufficient material was not available, but the writer found more than 16 chromosomes in heterotypic division. And, among the *Arosulatae*, *canina* even represents a hypo-octoploid type.

Which of the three *Rostellatae* groups is the more primitive is a difficult question. From a merely morphological point of view, one should suppose that the very little differentiated *Arosulatae* were the most primitive, and that the next one should be the *Rosulantes*, in which the main stem has been checked, and the flowers are developed on side stems from the axils of the leaves in the rosette. Finally, we should have the stemless *Scapigerae* as the last type, of which some still have preserved stems as supraterraneous runners in the service of propagation.

But against this speak several facts, especially the crossing possibilities as known from natural hybrids and from Gershoy's investigations (10). These, certainly, give *Rosulantes* a very central position among the *Rostellatae*. *Rosulantes* is the only group which can be crossed with *Chamaemelanium*, and it can be crossed with *Stolonosae*, *Adnatae* and *Borealiamericanae* of the *Plagiostigma* subsection, and with *Scapigerae* and *Arosulatae* of the *Rostellatae* (Fig. 59). The *Arosulatae* cannot even be crossed with *Scapigerae* of the same subsection; no natural hybrids are known, although the species very often grow intermixed in Europe. If *Arosulatae* should be the centre group or the prototype of the *Rostellatae*, one would expect them to be able to cross with the *Scapigerae*, but if both are divergent lines of development from a third prototype, this absence of crossing possibility could be accounted for.

The geographic distribution (Fig. 60) also supports the view that *Rosulantes* is the central group of the *Rostellatae*, as it is the only group which has representatives in America. If *Rostellatae* have originated from a central Pacific stock of *Chamaemelanium*, one really would expect its most central and primitive group to be represented in that part of the world. Which direction they travelled to Eurasia, west or east, is another question; *rupestris*, which is represented all over from Eastern America to Western Europe. must be supposed to have travelled westwards to Eurasia, as that American type, which most resembles the Eurasian ones, just grows in the Pacific region.

If the violets of the *Melanium* section have their prototypes in the

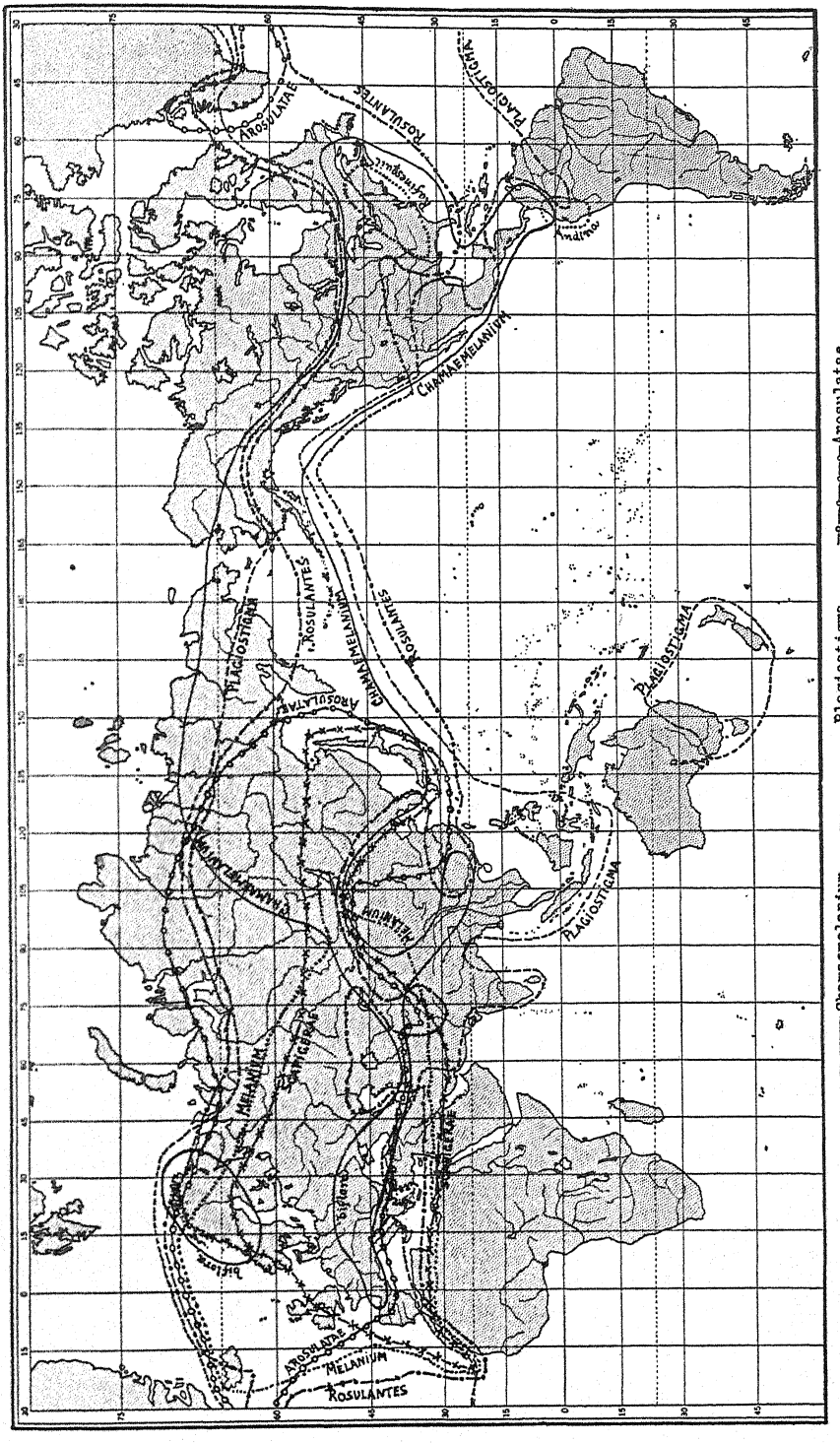


FIG. 60. Range of distribution of the main groups of the northern hemisphere violets.

Chamaemelanium section, one should think of *V. pedunculata* as one of the nearest now living relatives. No hybrids between *Chamaemelanium* and *Melanium* are known yet, but this species, no doubt, has not been tried for crossing. If the *Melanium* violets originated from some West American *Chamaemelanium* types, one must suppose they wandered eastwards. Peculiarly enough, only one indigenous *Melanium* violet is left in North America, viz. *V. Rafinesquii.* The main development of the section must have taken place in Europe or on the border line between Europe and Western Asia. Perhaps the crossings, which must be supposed to have taken place before all these types with their irregular series of chromosome numbers developed, were effected just in the area from the Balkan peninsula to Caucasia, where the greatest variation in *Melanium* species are found. It is difficult to tell which European species are the more related to *V. Rafinesquii.* At least, this species is more related to the *Tricolores* than to *Calcaratae*; the chromosome number ($n = 17$) also places it there. Of the two strictly annual pansies, *V. arvensis* and *V. Kitaibeliana*, it seems, irrespective of chromosome number, morphologically more related to some large-flowered *Kitaibeliana* types than to *arvensis.* This also is in harmony with the idea that *Kitaibeliana* is a comparatively primitive type in the *Melanium* section, and this idea again is supported by the fact that within the 'species' of *Kitaibeliana* apparently a great variety of chromosome numbers are found. The writer has isolated types from Caucasia with $n = 7$, 8, about 12 and 18 chromosomes, and recently one from Western Europe with $n =$ about 24, together with some hybrids with odd chromosomes; morphologically, all these were typical *Kitaibeliana.*

How the prototypes of the *Melanium* violets came to Europe we do not know. Wegener's theory about the continental dislocations, recently affirmed by measurement of time difference between Denmark and Greenland, might help us to explain the peculiar fact that one species of an otherwise totally European section is indigenous to North America. In this connexion it should be mentioned that if Becker's statement (4) is correct, we have still one American *Melanium* violet more, the South American Andine species, *V. andina* W. Beckr. The writer has never seen any specimens of this type.

The morphology, distribution, chromosome relations, and crossing possibilities of the violets, in this manner raise many problems and stir up many ideas, which hardly can be really substantiated, but still these conditions are suggestive, and some consideration of the possibilities might be justified, even if definite facts cannot be spoken of in all cases.

Summary.

1. The chromosome numbers of 13, mainly Pacific *Viola* species, have been determined (pp. 743–6).

2. Discrepancies between the countings of the present writer and those of Gershoy (10) have been considered.

3. In accordance with W. Becker (3) it is found most natural to treat *V. adunca*, J. E. Smith, as a subspecies of *V. rupestris*, Schmidt, which in this way becomes circumpolar (pp. 749–50).

4. A list of the 87 *Viola* species investigated for chromosome numbers is given (pp. 751–2).

5. A reclassification of the violets of the northern hemisphere is attempted, based upon morphological studies, and also upon what is known about chromosome numbers. The essential points of this reclassification are:

(*a*) The *Nominium* section has been divided in two subsections: *Rostellatae* with a 10-series of chromosome numbers, and *Plagiostigma* with a 12-series, and some minor aberrations from it.

(*b*) The former *Dischidium* section has been included in the *Chamaemelanium* section.

(*c*) The West American yellow violets, previously of the *Nominium* section, can very naturally be included in the *Chamaemelanium* section; *V. sarmentosa* is found to be nearly related to *V. biflora*.

6. Phylogenetic considerations. Morphological, cytological, genetical, and geographical principles have been used in order to attain a view regarding the phylogeny of the larger groups. The northern hemisphere temperate violets are thought to have originated from 6-series violets, which came up through Mexico from South America. The *Chamaemelanium* section is thought to be the primitive and central group with a great morphological variation recalling all the other groups. From some *Chamaemelanium* types the *Plagiostigma* subsection may have its origin, from others the *Rostellatae*, of which the most central and primitive group is thought to be the *Rosulantes*, and among still other *Chamaemelanium* types the *Melanium* section may have its ancestors.

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Studies in the Gramineae.[1]

VIII. On the Organization of the Flower in the Bamboo.

BY

AGNES ARBER, M.A., D.Sc.

With eight Figures in the Text.

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[1] For references to previous papers in this series, which represents the work carried out during the tenure of a grant from the Dixon Fund of the University of London, see 1–7 in the Literature cited, p. 781.

[Annals of Botany, Vol. XLIII. No. CLXXII. October, 1929.]

1. Introduction.

In former articles belonging to this series (1, 2, 4) I have described normal and abnormal structure in the flowers of a number of genera of the Bambuseae. Since these papers were written I have received a large amount of additional material, for which I am indebted to the kindness of the Director of 's Lands Plantentuin, Buitenzorg; the Superintendent of the Royal Botanic Gardens, Sibpur, Calcutta; the Director of the Royal Botanic Gardens, Kew; the Conservateur du Jardin des Plantes, Montpellier; the Director of the Botanic Garden, Singapore; the Conservator of Forests, F.M.S. and S.S.; and Dr. F. W. Foxworthy and Mr. E. J. Strugnell, of the Forest Research Office, Kuala Lumpur, F.M.S.

In the present paper I propose especially to consider certain trends in floral structure exhibited by teratological examples, as well as by genera which normally diverge from the fundamental flower-type of the Bamboos.

2. Abnormalities in *Bambusa* and *Schizostachyum*.

(i) *Bambusa nana*, Roxb.

Bambusa nana, the so-called Chinese Bamboo, is believed to be native to China and Japan, and its interest from our present standpoint lies in the fact that under cultivation elsewhere it is apt to show floral abnormalities such as those mentioned by Ruprecht (13) and Munro (11). The former figures it under the name of *Arundinaria glaucescens* (Pl. I, Fig. 3). Mr. H. N. Ridley, F.R.S., to whose help in studying this species I am much indebted, tells me that when it flowered at Kew, in 1862, the lower flowers were female and the upper hermaphrodite. The material which I have examined came from plants grown at Calcutta and in Java. In the specimens from the Buitenzorg Garden nearly all the spikelets I sectioned were normal. The structure is that of a typical Bamboo, with three lodicules, six stamens, and a gynaeceum with three stigmas (Fig. 1, A_1 and A_2, p. 767). In one spikelet alone was there any deviation from this plan. At its base the spikelet in question showed, enclosed in a prophyll, a lateral shoot which differentiated itself into four buds. Two of these were quite rudimentary; they were followed by two imperfect flowers, side by side, one of which was male, with three stamens only. Then, on the opposite side of the axis, there appeared an hermaphrodite but abnormal flower, which I will describe; it was succeeded by seven normal flowers, and an eighth which was rudimentary. The peculiarity of the abnormal flower was that the back lodicule was united with a back lateral stamen (Fig. 1, B_1 and B_2, p. 767) and the gynaeceum with the back median stamen (B_2–B_4). Or, in other words, the back lodicule and the wall of the gynaeceum each bore an anther. Despite this intimate

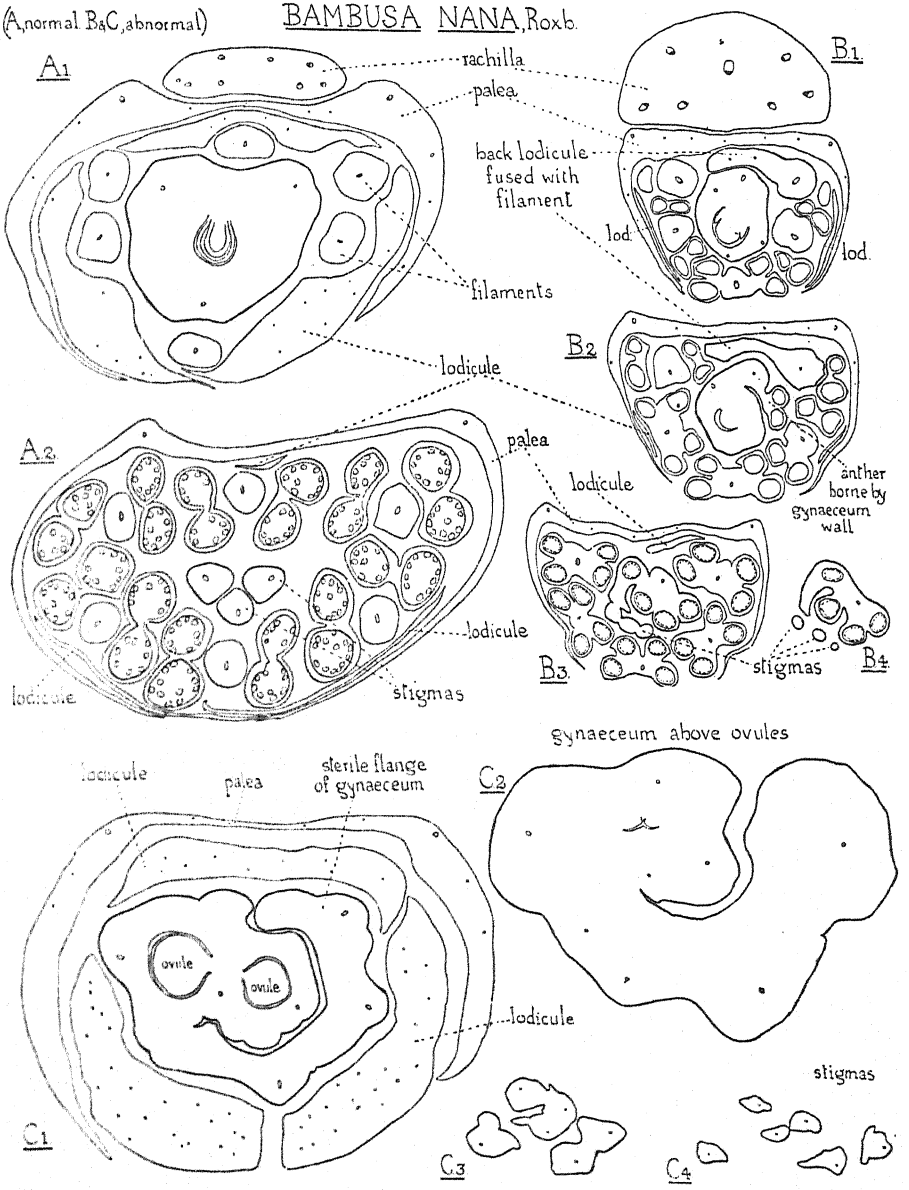


FIG. 1. *Bambusa nana*, Roxb. All diagrams from transverse series taken from below upwards through spikelets (× 48). A_1 and A_2, normal spikelet, Buitenzorg Garden, September 1927. Lemma omitted in both, and rachilla omitted in A_2; A_1 passes through filaments and ovary, and A_2 through anthers and stigmas. B_1–B_4, from an abnormal flower, younger than that drawn in A, Buitenzorg Garden, September 1927. In B_4 only the stigmas and the gynaeceum-stamen are shown. For description see text, pp. 766 and 768. C_1–C_4, a biovulate female flower from the Calcutta Gardens; series passing from C_1, ovary, to C_4, stigmas. In C_2–C_4, only the gynaeceum is shown.

association with a stamen, the gynaeceum was normal and complete, with one ovule (B_1) and three stigmas (B_4). There were four normal stamens in addition to the two which were involved in these fusions.

The spikelets which I received from the Calcutta Gardens were collected in three different years. They agreed among themselves, and differed from the Buitenzorg spikelets, in that they contained female flowers exclusively. In one flower alone there was a circle of six minute rudiments, evidently representing filaments, surrounding the base of the ovary, but I noticed neither normal nor vestigial stamens in any other flower. The Superintendent, however, informs me that flowers with stamens also occur in the Gardens. In the female spikelets I found a tendency to abnormality in the gynaeceum. Some of the ovaries contained the single ovule normal for the Gramineae, while others were biovulate. In the younger ovaries it is not always easy to distinguish the ovules, but in thirty-eight flowers in which their number could be determined with some certainty, seventeen ovaries contained one ovule, while twenty-one contained two. A biovulate gynaeceum is shown in Fig. 1, C_1. It will be seen that the two ovules are turned to right and left, and it is remarkable that in ovaries in which there is only one, it also often occupies a lateral position, as though it were one of a pair. The gynaeceum of the flower figured terminates above in six stigmas (C_4). The number of stigmas is highly variable. In thirty-four flowers in which I counted them, I found three stigmas in one flower; four in five flowers; five in twelve flowers; six in nine flowers; seven in two flowers; eight in one flower; eight, subsequently branching to nine, in one flower. Five and six are thus the prevalent numbers.

(ii) *Bambusa lineata*, Munro.

Bambusa lineata is native to the marshy coast forests of the Malay Archipelago and the Moluccas (10). It is cultivated in the Buitenzorg Garden, and flowers from this source which I examined had the structure normal for the species (Fig. 2, A_1, A_2, p. 769) with three lodicules and six stamens—a typical equipment for a Bamboo. But specimens from the Calcutta Garden showed flowers which were reduced both as regards lodicules and androecium. It should be noted that Gamble (10) reported that he had failed to find lodicules in this species. The flower drawn in Fig. 2, B_1–B_3, p. 769, possessed two lodicules; the back member was absent, while one of the front pair occupied a peculiar position, from its very base enwrapping the filament of one of the front stamens (B_1). The three stamens of the outer whorl were normally developed, while the three of the inner whorl were all abnormal (B_2). The back one had a wide connective and two pollen-sacs only; it may possibly represent the back stamen, and the missing back

lodicule, in a state of union. One of the front lateral stamens had two pollen-sacs, while the other was reduced to a non-vascular filament with no anther. The three normal stamens were taller than the three others (B_3).

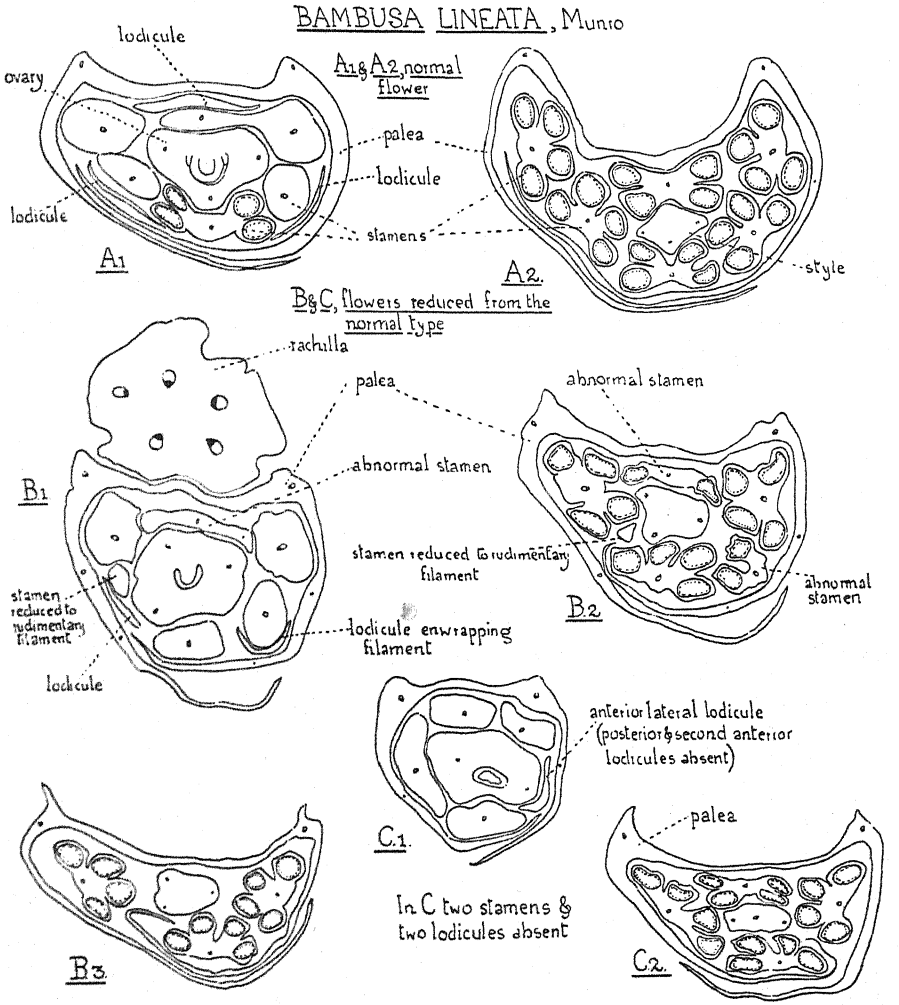


FIG. 2. *Bambusa lineata*, Munro. All diagrams from transverse series passing upwards from below through spikelets (× 62). A_1 and A_2, normal form from Buitenzorg Garden; in A_1 the section passes so near the flower-base that two of the filaments are united. B and C, abnormal forms from the Calcutta Gardens; the drawings B_1–B_3 are generalized from more than one section. For descriptions of B and C, see text, pp. 768 and 769.

In C_1 and C_2 a flower belonging to a second spikelet from Calcutta is drawn. Here two stamens are absent—presumably the front members of the inner whorl—and only one of the front lateral lodicules is developed (C_1).

(iii) *Schizostachyum chilianthum*, Kurz.

I have figured a normal flower of *Schizostachyum chilianthum* from the Federated Malay States, where it is native, in 1, p. 463, Fig. 10; it has three lodicules and six stamens. In a spikelet from a plant cultivated in the Buitenzorg Garden I have since found the abnormality drawn in Fig. 3. The back lodicule and three of the stamens are normally developed,

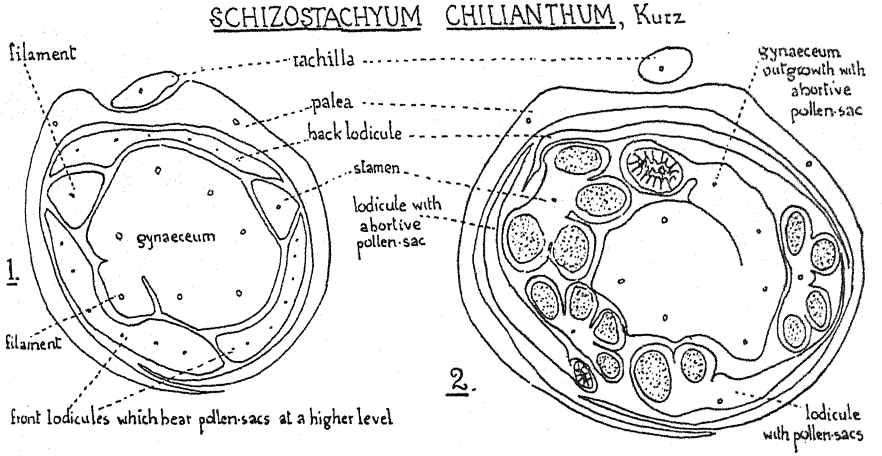


FIG. 3. *Schizostachyum chilianthum*, Kurz. Two transverse sections, 1, lower, and 2, higher, in a series passing through a spikelet from a plant cultivated in the Buitenzorg Garden (× 62). In both, only the main bundles are shown in the palea, and in 2, which is generalized from several sections, the vascular strands of the lodicules are omitted.

but the two front lodicules bear pollen-sacs, while the back inner stamen and two others are absent. Moreover, the gynaeceum bears an outgrowth with an abortive pollen-sac occupying the place of the back stamen. This flower thus contains both stamen-lodicules and a gynaeceum-stamen.

3. THREE-STAMENED BAMBOOS.

In the three teratological cases just described, the lodicule-androecium system shows a tendency to abnormality and reduction, which specially affects the inner whorl of the androecium. We now pass on to the three-stamened genera which are grouped together as the sub-tribe Arundinarieae, in contradistinction to the six-stamened Eubambuseae. In the Arundinarieae the disappearance of the inner stamen-whorl, foreshadowed by abnormal cases in the Eubambuseae, has become an accomplished fact. I have examined five of the three-stamened genera, but in all cases I have had herbarium material only.

(i) *Phyllostachys*.

In 1921–2 *Phyllostachys aurea*, Rivière, flowered at Montpellier, and M. Jules Daveau, who has himself described the flowering (9) has been so

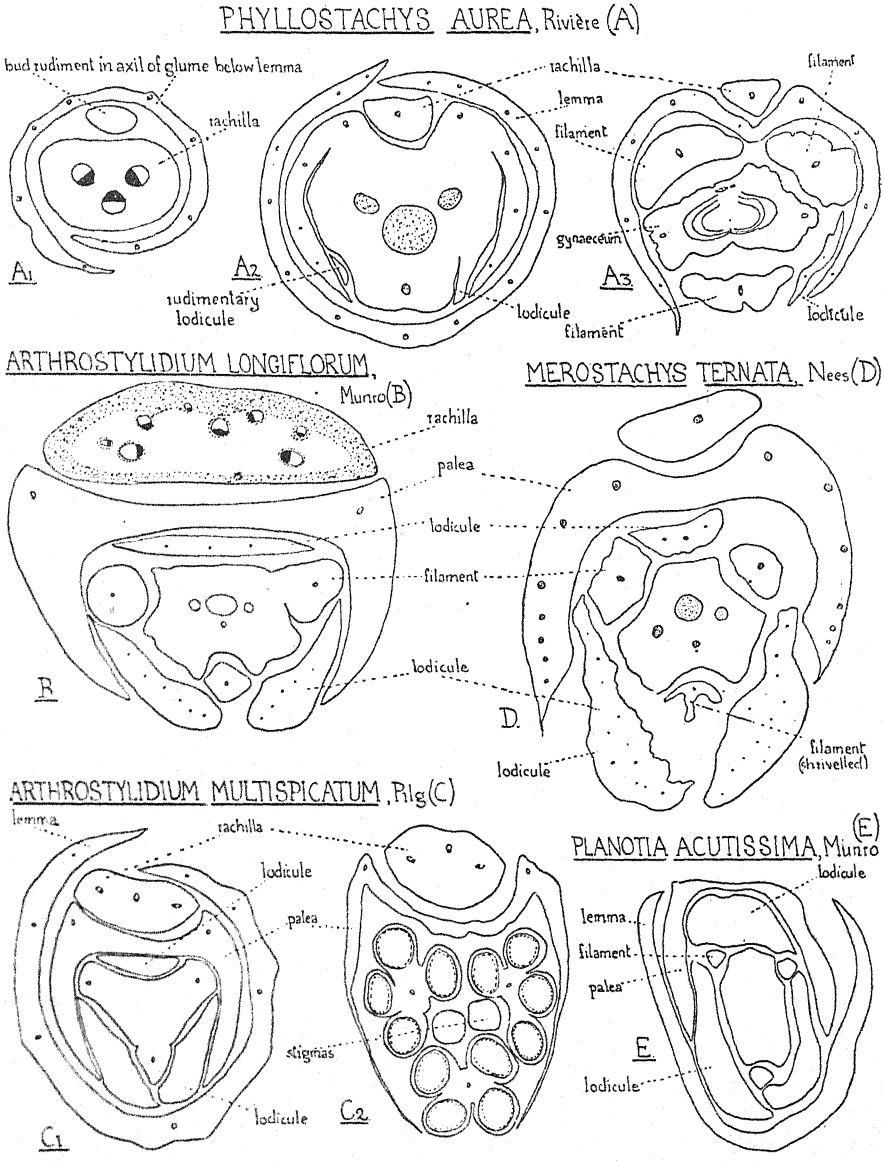


FIG. 4. Three-stamened Bamboos. All diagrams drawn from transverse sections from series from below upwards through spikelets (× 47, except C_1 and C_2, which are × 77). A_1–A_3, *Phyllostachys aurea*, Rivière, from Botanical Garden, Montpellier; spikelet from shoot put out from rhizome after cutting down of the flowering haulms. B, *Arthrostylidium longiflorum*, Munro, Colombia: more than one section used. Note lack of radial symmetry in the rachilla. C_1 and C_2, *Arthrostylidium multispicatum*, Pilger. D, *Merostachys ternata*, Nees, Brazil. E, *Planotia acutissima*, Munro, Colombia.

kind as to send me herbarium material of spikelets. In the axil of the glume below the lemma (flowering glume) I found a rudimentary bud, which is shown in Fig. 4, A_1. Such a bud occurred in two of the three spikelets

which I cut, while the third series did not include this region. These rudimentary buds may be compared with those in the axils of the glumes below the lemma in *Streptochaeta* (6, Fig. 2, C_2, p. 38, and Fig. 4, C, p. 40). M. Daveau has noted that the lodicules, where they exist, are always rudimentary, and my observations confirm this. Fig. 4, A_2, shows a small rudimentary front lodicule on the left, and a slightly more developed one on the right. In a second spikelet from Montpellier I again found two front lodicules and no back one. Two spikelets from South Devon, where this Bamboo had flowered under cultivation, were given to me by the late Mr. Gamble; neither possessed a back lodicule, but one had both front lodicules, and the other only one.

(ii) *Arthrostylidium.*

I have drawn *A. longiflorum*, Munro, from Colombia (Fig. 4, B, p. 771) and *A. multispicatum*, Pilger (Fig. 4, C_1 and C_2, p. 771), as examples of the general structure of the genus. *A. Prestoei*, Munro, is similar. The lodicules in all these three species emerge between the lips of the palea. The section of *A. longiflorum* (Fig. 4, B) is remarkable for the approach to dorsiventral rather than radial symmetry in the rachilla; in a second spikelet the same feature was recognizable, though it was less pronounced. The ground-plan of the rachilla in this species thus recalls that of the branches of the first order in the inflorescence of *Luziola Spruceana*, Benth. (5, Fig. 2, G, p. 395).

(iii) *Merostachys.*

Fig. 4, D, p. 771, represents a transverse section of an old flower of *Merostachys ternata*, Nees; it closely resembles another Brazilian species, *M. speciosa*, Spr., of which I have also cut sections. The shrivelled state of the front filament in Fig. 4, D, suggests that the stamen may possibly be abortive; it may be noted in this connexion that Nees (12) speaks of finding only two stamens in *M. speciosa*.

(iv) *Planotia.*

The three filaments and three relatively large lodicules of *P. acutissima*, Munro, from Colombia, are shown in Fig. 4, E. *P. virgata*, Munro, from Trinidad, and *P. nobilis*, Munro, from New Granada, do not differ essentially from *P. acutissima*.

(v) *Chusquea.*

Among the Bamboo genera which I have examined *Chusquea* is remarkable for the stamen-like character of the base of its back lodicule. In sections cut near the level of origin of this lodicule in *C. Cumingii*, Nees, from Chili (Fig. 5, A_1, p. 773) and *C. nigricans*, Phil., from Valdivia (Fig. 5, B),

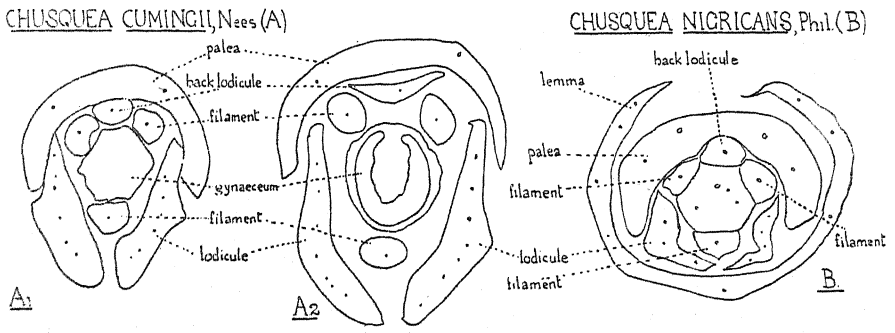


FIG. 5. *Chusquea.* Diagrams of sections from transverse series from below upwards through spikelets (× 47). A_1 and A_2, *C. Cumingii*, Nees, from near Valparaiso, Chili; A_2, from more than one section. B, *Chusquea nigricans*, Phil., from Valdivia.

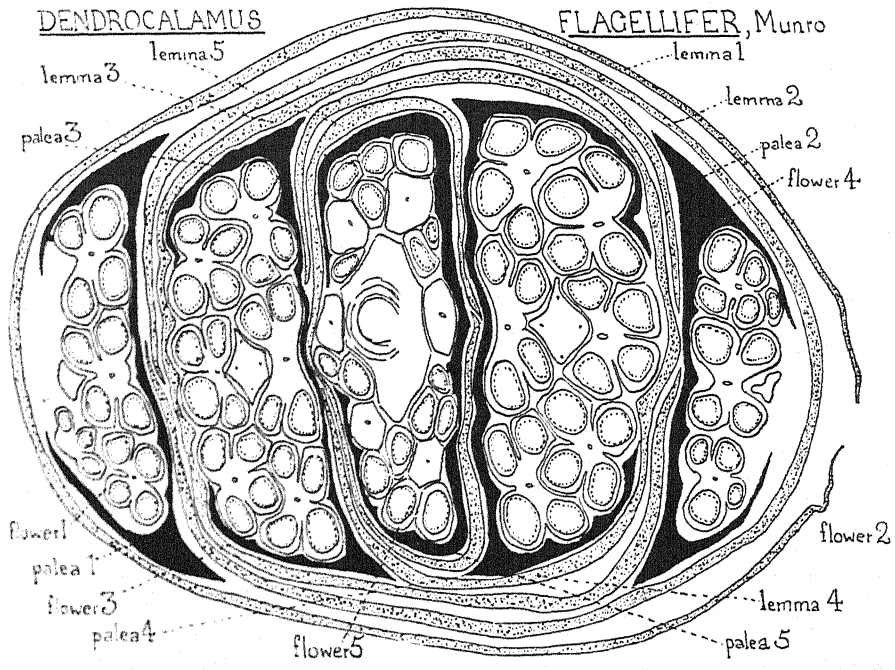


FIG. 6. *Dendrocalamus flagellifer*, Munro (collected by F. W. Foxworthy, Perak). Transverse section (× 62) from a series through a young spikelet, showing five flowers; two outermost glumes somewhat broken and reconstructed from other sections lower in the spikelet. Several of the stamens in the lowest flowers (1 and 2) do not reach to the level of the section. Flower 5, the highest, is cut through the ovule, and the other flowers through the styles. Lemmas dotted and paleas indicated in solid black.

its outline and its single bundle make it look deceptively like a filament. A little higher up, however, it becomes wider and flatter (A_2). Its base shows the same staminal character in *C. pinifolia*, Nees, from Brazil.

4. The Spikelet of *Dendrocalamus*.

The Bamboos we have hitherto considered have all shown (either as a normal or abnormal character) some degree of reduction in the lodicule-androecium system, from the three lodicules and six stamens which we may consider fundamental for the tribe. In Fig. 6, p. 773, I have drawn a transverse section of a spikelet of *Dendrocalamus*—a genus in which there are six stamens, but, as a rule, no lodicules. This diagram illustrates the tight packing of the individual flowers with which the loss of lodicules may be correlated. For clearness, the lemmas (flowering glumes) are dotted and the paleas indicated in solid black.

5. Flower Structure in *Ochlandra*.

(i) *Introduction.*

Nearly all the variations in the lodicule-androecium system of the Bamboo flower dealt with up to this point have followed the direction of reduction from the type with three lodicules and six stamens. A different evolutionary path is illustrated by *Ochlandra*, a singular genus occupying an isolated position among the Bambuseae. The one-flowered and often large spikelets are characterized by a great range in the number of stamens (6–120), a variable number of lodicules, and from three to six stigmas. I have long wished to make a full comparative examination of this aberrant genus, but material for an exhaustive study seems unattainable, and the present account relates only to the four species which I have been able to procure out of about ten which are recognized. By the kindness of the Director of 's Lands Plantentuin, Buitenzorg, I have received well-fixed spikelets of *O. setigera*, Gamble; in the case of the other species I have had to use dried material.

(ii) *Ochlandra setigera*, Gamble.

In 1927 *O. setigera* flowered freely in the Buitenzorg Garden. The Director tells me that in December of that year the seeds were 'heaped in hundreds' under the plant. When Gamble described this Bamboo (10, p. 128) the flowers were unknown, so he treats of its vegetative characters only, but Mr. H. N. Ridley, who has kindly examined the Buitenzorg plant, considers that it certainly belongs to this species. Fig. 7, A_2, p. 775, shows the general construction of a very young spikelet, the glumes below the lemma being omitted. The axis is continued beyond the flower as a small rachilla which is enwrapped by the lemma. The palea is not bikeeled. This particular flower had five lodicules and twenty-five stamens (one with six pollen-sacs), but in another spikelet I found seven lodicules and thirty-two stamens. A striking feature of the flower is that the filaments, from

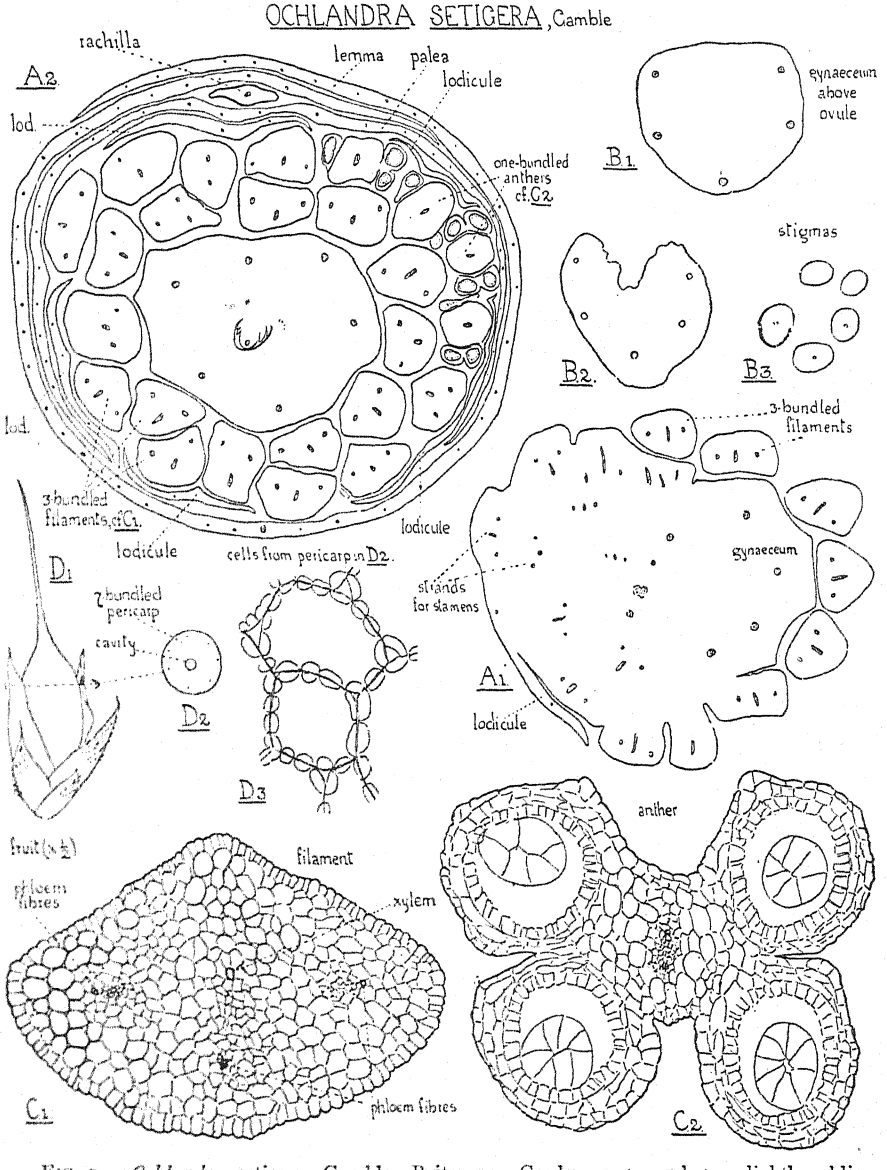


FIG. 7. *Ochlandra setigera*, Gamble, Buitenzorg Garden. A_1 and A_2, slightly oblique transverse sections from a series from the base upwards through a young flower (× 47). A_1, base of ovary and associated structures only. B_1–B_3, passage from top of ovary, which was six-bundled below B_1, to stigmas (B_3), continuing the series in A, but from transverse sections of another flower, on a larger scale (× 77). C, transverse sections through filament (C_1) and anther (C_2) of a stamen (× 193). D_1, fruit enclosed in glumes (× ½). D_2, transverse section of fruit at level of arrow (× ½). D_3, ground-tissue cells from D_2 (× 193); the starch grains which filled the cells are omitted.

their bases up to the level of attachment of the anther, possess three bundles —a median and two smaller laterals. That this three-bundled character originates below the base of the stamen, and is not due to branching of a single strand within the filament, is proved by Fig. 7, A_1. In this section, which passes with slight obliquity through the base of the gynaeceum, the stamens to the left have not yet freed themselves, while those to the right are detached. It will be seen that before the filaments leave the flower-base they are each provided with three bundles. The anatomy of the stamen is shown in greater detail in C. The filament is drawn in C_1; the laterals possess no lignified xylem, but have one or two conspicuous phloem fibres. In C_2 the anther has become one-bundled owing to dying out of the laterals.

The young gynaeceum in Fig. 7, A_2, has six strands in its thick pericarp, in addition to ovule strands which can only be faintly discerned. In B_1–B_3 the further history of such a six-bundled gynaeceum can be followed from another flower. As the gynaeceum is traced upwards, there is a tendency to loss on the posterior side, due probably to the compression which in so many Grasses affects the face of the flower turned towards the rachilla. The first sign of this is the loss of the posterior bundle, which has already occurred in B_1: the style becomes grooved on this side (B_2), and finally breaks up into five stigmas, of which the two posterior members are less developed than the three anterior. In Figs. 8, A_1–A_3, and B_1–B_3, p. 777, seven-bundled gynaecea can be seen giving rise to five and four stigmas respectively. Fig. 8, A_3, is also interesting as a case in which the rachilla, instead of dying out above the functional flower, gives rise to a rudimentary flower, and then again becomes lobed at its extreme apex.

The fruit of *O. setigera* is drawn in Fig. 7, D_1, p. 775). It is relatively large: the diameter of the example sketched was over 1·5 cms. The pericarp is extremely solid (D_2), and sections show that its ground-tissue consists of cells with thick, pitted walls (D_3), which are crowded with starch grains.

(iii) *Ochlandra Beddomei*, Gamble.

I have been able to examine one dried spikelet of this species from Wynaad, South India, given to me by the late Mr. Gamble; it dates from 1875–6, the only known flowering period of this Bamboo (8). The spikelet contained a mature flower of which the anthers had already dehisced. The flower was lateral to a small rachilla, which, however, did not reach to the level of the section drawn in Fig. 8, C_1, p. 777, which traverses the filaments. I found four lodicules and thirty-one stamens, one of which had six pollen-sacs, and so possibly represented a fused pair; Gamble, in his description, gives 'about 32' as the number (10). The filaments are remarkable in that they contain five bundles (C_2): sometimes there seem to be even more, but the condition of the material makes counting difficult. The asymmetry in

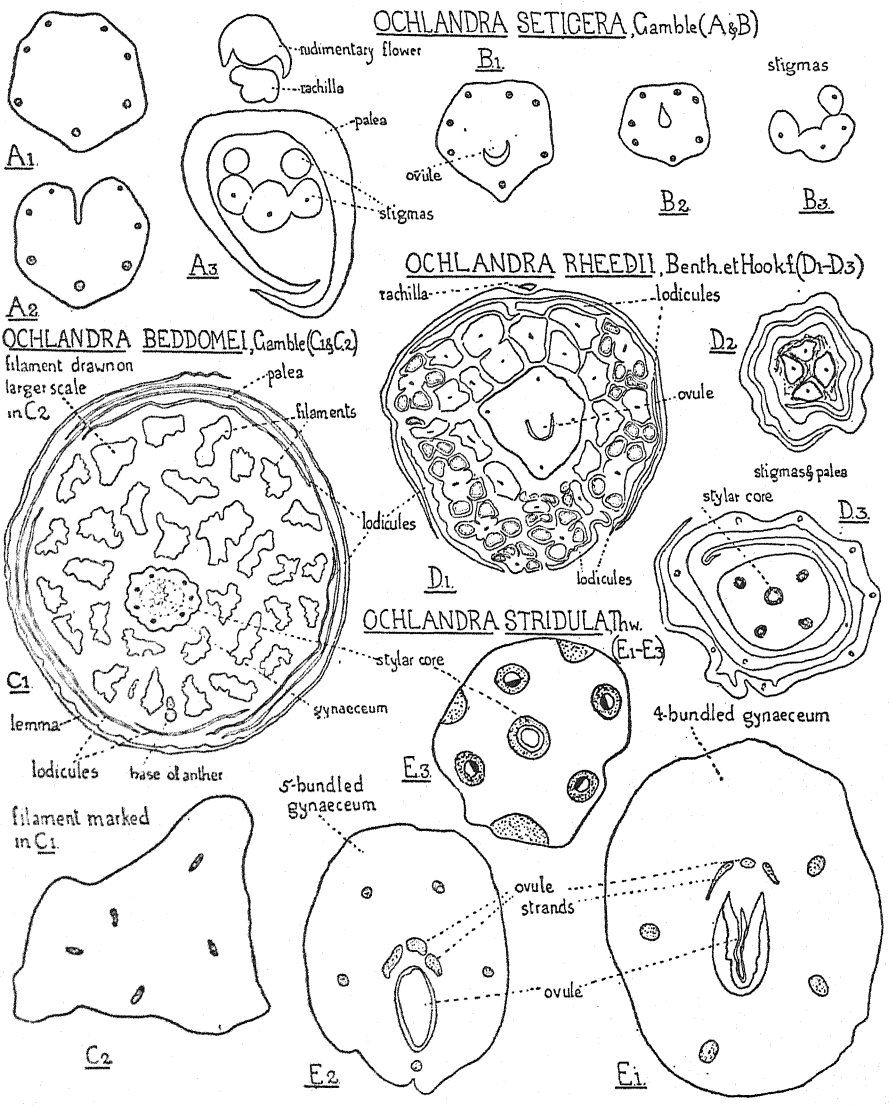


FIG. 8. *Ochlandra.* A and B, *O. setigera*, Gamble, Buitenzorg Garden. A_1–A_3, sections (× 77) from a transverse series from below upwards through the top of a seven-bundled gynaeceum to show origin of five stigmas. A_3, includes palea and rachilla, and shows development of a rudimentary flower above the normal flower. B_1–B_3, sections (× 77) from a transverse series from below upwards through a very young seven-bundled gynaeceum to show origin of four stigmas. C, *O. Beddomei*, Gamble; C_1, transverse section (× 14) through a spikelet above level of top of rachilla, passing through filaments and gynaeceum above ovule. C_2, transverse section of the filament marked in C_1 (× 77). D, *O. Rheedii*, Benth. et Hook. D_1 and D_2, transverse sections (× 47) from a flower (Munro, Cochin). D_1 passes through the rachilla and through the ovary at the level of the ovule; some stamens cut through filament and some through anther. D_2, four stigmas. D_3, upper part of gynaeceum of another flower (× 47) to show fibrous sheaths of bundles and stylar core. E, *O. stridula*, Thw. (Ceylon); E_1 and E_2, transverse sections through two ovaries, E_1 (× 23); E_2 (× 47). E_3, transverse section (× 77) through upper part of a gynaeceum.

Fig. 8, C_2, is due also to the imperfect recovery of the tissues of this dried flower. When the stamens have once elongated, the tenuous substance of their filaments readily contracts on drying, so that it is difficult to get good sections. The gynaeceum wall consists of a firm outer layer enclosing seven bundles; then a zone which appears to have been lacunar, and in which only relics of tissue remain; and in the centre a firm core, probably enclosing the conducting tissue of the style. In the only existing figure showing a transverse section of the flower of an *Ochlandra*—Schuster's drawing of *O. travancorica* (14, Fig. 29, p. 247)—this author appears to have altogether misunderstood the structure, for he describes the central object as a stamen-tube fused with the gynaeceum, whereas comparison with my Fig. 8, C_1, leaves little doubt that it is the gynaeceum alone—the nine bundles (which he regards as the vascular supply for the 120 anthers) being on my interpretation the ring of bundles in the gynaeceum wall. The zone which he describes as fused lodicules I take to be the tube formed by the filaments, which in this species are monadelphous.

(iv) *Ochlandra Rheedii*, Benth. et Hook.

I have examined several spikelets named as *O. Rheedii*, though their general structure does not altogether bear out the description of this species. The ground-plan of the spikelets (Fig. 8, D_1) recalls that of *O. Beddomei*. There were five lodicules in the flower illustrated, and in a second I found five, while another had six, one of which was unusually wide. In two flowers I found twenty-four stamens; in the one drawn there was some indication that these stamens were in two rings of twelve each. A third flower had seventeen stamens, and another appeared to have twenty-two. In two of the flowers examined, at least three bundles could be distinguished in the filaments, but the preservation was not good enough to make an exact count possible. It will be seen from D_1 that there are four bundles in the gynaeceum wall, fibres being associated with these strands as the gynaeceum gets older (D_3). There are four stigmas (D_2).

(v) *Ochlandra stridula*, Thw.

In *O. stridula* the rachilla continues above the flower as in the other species described. My specimens were too old to retain more than the relics of the androecium, but one or two filaments in which I could see the basal structure showed more than one bundle. The age of the flowers had, however, the advantage that the vascular skeleton of the gynaeceum was better developed than in the other species studied. In four spikelets in which I could follow the anatomy, there were four bundles in addition to the ovule-strands in three flowers (E_1) and five in one flower (E_2). The upper

part of a gynaeceum is drawn in E_3 to show the sclerized character of the bundle-sheaths and the marked asymmetry of their arrangement, and of that of the superficial fibrous patches.

6. Conclusions.

On reviewing the observations detailed in this paper, one is struck by the fact that all the abnormal features illustrated for *Bambusa nana* (Fig. 1, p. 767), *B. lineata* (Fig. 2, p. 769) and *Schizostachyum chilianthum* (Fig. 3, p. 770) can be paralleled among those previously recorded for *Cephalostachyum virgatum* (2) and *Schizostachyum latifolium* (4). In the first place, in each of these five Bamboos, 'stamen-lodicules' (structures transitional between lodicules and androecium members) have been found. The lodicule with pollen-sacs to the right in Fig. 3, 2, p. 770, which represents these structures in *Schizostachyum chilianthum*, Kurz., may be compared with one of the stamens with lodicular wings in 4, Fig. 4, A_1, p. 183 (*Schizostachyum latifolium*, Gamble) and with *a* in 2, Fig. 3, C_4, p. 55 (*Cephalostachyum virgatum*, Kurz.). Moreover, the reduction in the inner whorl of stamens observed in *B. lineata* (Fig. 2, p. 769) recalls the tendency to reduction of the same whorl noticed in *Cephalostachyum virgatum*; for example, the stamen reduced to a rudimentary filament in Fig. 2, B_1, B_2, p. 769, may be compared with a similar structure in 2, Fig. 7, E, p. 66. In *Bambusa nana* the tendency to stamen reduction may go so far as to result in entirely female spikelets. The three-stamened Bamboos drawn in Fig. 4, p. 771, and Fig. 5, p. 773, illustrate the stage in which the loss of the inner androecium whorl can no longer be regarded as teratological, since it has become stereotyped as a generic character; this stage is foreshadowed by the partial abortion of this whorl in such examples as the abnormal flower of *Bambusa lineata* drawn in Fig. 2, B_2, p. 769. But even in the three-stamened Bamboos things are not stationary in the lodicule-androecium system. We find, for instance, in *Phyllostachys aurea* (Fig. 4, A_2 and A_3, p. 771) that the back lodicule, and sometimes another, may be absent. As hinting at the close relationship of lodicules and stamens, we may notice the filament-like structure of the base of the back lodicule in *Chusquea* (Fig. 5, A_1 and B, p. 773).

It seems reasonable to attribute the tendency to reduction, which is such a marked feature of the lodicule-androecium system in the Bamboos described, to the extremely tight packing between the glumes to which the flowers of so many Gramineae are subjected in the bud stage. Fig. 6, p. 773, illustrates this point for *Dendrocalamus*, a genus in which lodicules are generally absent. The effect of pressure in causing reduction of the stigmas of *Ochlandra* is shown in Fig. 7, B, p. 775 and Fig. 8, A and B, p. 777.

In carpels as well as stamens we meet with a close relationship in teratological features between *Cephalostachyum virgatum*, *Bambusa nana*,

and *Schizostachyum chilianthum*. In all three we are liable to find an outgrowth from one side of the ovary wall, more or less enwrapping it, and giving a suggestion of spiral form to the transverse section of the organ. The diagram showing this sterile flange or lobe from the gynaeceum of *Cephalostachyum* in 2, Fig. 3, C_1, p. 55, could be paralleled from among my preparations of *Bambusa nana*. Further, the gynaeceum flange, instead of being a lobe of merely sterile tissue, may bear one or more pollen-sacs, and the whole structure may then be called a 'gynaeceum-stamen'. These curious organs, like the sterile lobes, repeat themselves in the different genera with which we are concerned. The diagram of a gynaeceum outgrowth with an abortive pollen-sac in *Cephalostachyum* (2, Fig. 5, D, p. 63) closely resembles the gynaeceum-stamen of *Schizostachyum chilianthum* drawn in Fig. 3, 2, p. 770; while for the case of gynaeceum-stamens in which the pollen-sacs are normal, there is an exact parallelism between *Bambusa nana* and *Cephalostachyum* (cf. Fig. 1, B_2 and B_3, p. 769, with 2, Fig. 6, A_3 p. 65). The recurrence of this very definite teratological form must have some significance, and if the clue to it could be seized, it might throw light on the nature of the gynaeceum in the Gramineae—but at present its meaning eludes me.

In addition to the gynaeceum-stamen, abnormalities involving the ovules and the stigmas recur in more than one of the Bamboos studied. We may find in the ovary two ovules, instead of the single one which is universally characteristic of the Gramineae. These biovulate gynaecea have been observed in *Bambusa nana* and in *Cephalostachyum virgatum*, and they are closely similar in these two species (cf. Fig. 1, C_1, p. 767, with 2, Fig. 8, A, p. 70). Increase in number of the stigmas is another feature common to certain abnormal flowers in both these Bamboos, but it is carried to a further point in *Bambusa nana* than in *Cephalostachyum*; in the latter I have found flowers with four and with five stigmas, but none with higher numbers, whereas not only four and five, but even six, seven, eight, and nine have been seen in *Bambusa nana*. The increase in the stigmatic number, which is found as an abnormality in these two Bamboos, occurs as a normal feature in species of *Ochlandra*. The same relation between a teratological and a normal character is seen in the reduction of the inner stamen-whorl, which, occurring as an abnormality in certain of the six-stamened Bamboos, reappears as a fixed character in the Arundinarieae.

It will, I think, be admitted from the examples adduced that there is a definite parallelism in floral abnormality between the five Bamboos which we have discussed, though they represent three genera belonging to widely different sections of the Bambuseae; and, moreover, it seems clear that some of the abnormal features which they present recur as specific or generic characters elsewhere in the tribe. The conclusion to which these considerations lead is that abnormalities are by no means so casual and erratic as

is sometimes assumed. There is a tendency among morphologists to treat teratological cases as 'anomalous' (i.e. 'outside the law') while normal structure is regarded as being fixed in accordance with the laws of organic form. But though deviations from the normal are liable to seem chaotic at first glance, their analytical study reveals an underlying regularity, less patent, indeed, than that which is so readily discerned in normal examples, but no less inevitable.

7. Summary.

In this paper, which carries further the study of the Bamboo flower begun in previous articles of this series (1, 2, 4) various abnormalities are described—'stamen-lodicules', reduced androecia, 'gynaeceum-stamens', and increase in the number of ovules and of stigmas. Attention is drawn to the parallelism which exists between abnormal development in different Bamboos, and it is shown that certain features which are teratological in some genera, become normal characters in other sections of the tribe.

Among points of detailed structure recorded, reference may be made to the multifasciculate character of the stamen-filaments in *Ochlandra* (Fig. 7, A_1, A_2, C_1, p. 776, and Fig. 8, C_2, p. 777). I know of no other member of the Gramineae in which the filament is supplied by more than one bundle.

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Investigation of a Mendelian Ratio in Vigna sinensis by a Consideration of the Progeny from Successive Daily Crosses.

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With seven Figures in the Text.

THE general consideration of this series of experiments was suggested by Dr. S. C. Harland while the authors were working at the Imperial College of Tropical Agriculture, Trinidad, B.W.I., the original idea being to determine whether, by analysing an F_2 population from a single factor cross into the progenies of each day's flowering of the F_1 generation, any significant departure from the expected Mendelian ratio would appear.

The analysis of the F_2 generation as set forth in Table I of the present paper has already appeared (4). The interpretation here set forth carries the analysis a stage further and gives for the first time a detailed account of the experiments.

Previous workers in genetics have found in considering an F_2 population that for some reason, hitherto unexplained satisfactorily, the obtained ratios in some cases varied significantly from the expectation on the lines of simple Mendelian inheritance. Doubtless many of these departures can be attributed to a population of insufficient size or to defects in technique, but some have certainly been significant.

Previous Work.

Zederbauer (8, 9, 10) was the first worker to consider the possible influence of *parental age* on resulting progeny. He experimented with *Pisum*, using the varieties 'American Wonder' and 'Anslôs de Grâce', and studied the colour (yellow or green) and the appearance (smooth or wrinkled) of the seed coat. In his first paper on the subject he suggested the possibility of divergences appearing when individuals of different 'phase of life' were crossed. Zederbauer defined 'phase of life' of a flower as the

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position of the flower on the plant diagram, that is by its height on the stem, or, more definitely, by its node-number. Thus a cross between flowers of dissimilar 'phase of life' would be obtained by hybridizing a flower in some definite position on one plant by pollen from a flower in a different position on another plant. These he called *Heterochronic crosses.* Crosses between flowers at the same height on the two plants, i. e. between flowers of similar phase of life, are called *Isochronic crosses.* Experimenting with Iso- and Heterochronic crosses between the two varieties mentioned, Zederbauer reached the following conclusions:—

1. Heterochronic crosses have a greater liability to fail than Isochronic crosses.

2. Productiveness declines with age. An actual survey of his figures shows that the mean number of seeds per pod rises slightly from the first-produced pods to those of the medium stage in the development of the plant, and subsequently decreases to the later-produced pods.

3. His third and most important conclusion is that during the entire period of the plant's life the valence of a character changes from high valent to low valent: that in the later period of the plant's development there is an increasing tendency for recessive and subvalent forms to be produced.

Kappert (5) in a paper published in 1922 gives the results of a series of experiments on the same lines as those of Zederbauer, using the same varieties 'American Wonder' and 'Anslôs de Grâce'. He made several crosses and obtained 21 seeds from Isochronic crosses, 14 seeds from Heterochronic crosses in which a late-produced flower was crossed by an early-produced flower, and 45 seeds from Heterochronic crosses in which an early-produced flower was the female parent. The resultant F_1 generation (of 70 plants) gave 4485 seeds, which were collected and mixed indiscriminately and sown. The results obtained gave a deviation from a 3 : 1 expectation *not* greater than the standard deviation and therefore not significant. This held true for both the characters studied, (*a*) colour and (*b*) appearance of the seed coat. He further investigated Zederbauer's claim to the production of recessive and subvalent forms in excessive numbers from the seed set during the later stages of the plant's life. He collected seed from equally old pods and examined the plants produced therefrom, concluding that the deviation from expectation on a 3 : 1 basis was within the standard deviation, and refuting Zederbauer's results. In his discussion and criticism of Zederbauer's work Kappert attributes the aberration of ratios to insufficient castration.

It would seem that the value of Kappert's work is vitiated by his action in mixing together the seed obtained from his three types of cross. He had obtained Isochronic crosses, and two types of Heterochronic cross, one on each side of what we may call Isochronism. From the

progeny of these three distinct classes, lumped together, the deviation from Mendelian expectation was not significant. Whatever deviations might occur by virtue of the difference in 'phase of life' would tend to compensate one another by mixing the classes.

Meurman (6) published the results of a series of experiments on varieties of *Pisum*, using varieties different from those of Zederbauer and Kappert. His results appear to run parallel to and to bear out those of Kappert. He found no difference in F_1 and F_2 between seeds produced from Isochronic and Heterochronic crosses, or, as he states, no more than that found between young, old, and medium pods with respect to any of the characters of the F_2 population. He also discusses Zederbauer's work, and his criticism, like that of Kappert, is based on insufficient castration of the material.

The Present Investigation.

The present work was undertaken on the Experimental plots of the Imperial College of Tropical Agriculture, Trinidad, and the cowpea, *Vigna sinensis*, was selected as the most suitable material. It is a plant of reasonable size, is comparatively hardy and disease-free, and is easy to cultivate and handle. The floral parts are comparatively large and unlike, for example, *Dolichos lablab*, it is readily hybridized by the ordinary methods. It is normally self-fertilized, but Harland (2) suggests that a certain amount (up to 5 per cent.) of natural crossing may occur through the agency of the Carpenter bee (*Xylocopa* sp.). The plant is very prolific, and the pods contain from 8 to 12 seeds. The time from planting to maturation of pods is about 10 to 14 weeks, and thus three generations can be grown within a year. Most varieties are short bushy plants; some have an indeterminate habit of growth, but this is a simple recessive to the determinate habit.

For the purpose of the present experiment a plant with the above general characters was very suitable, but in addition there was required a definite and distinct character which would be related to its absence by simple Mendelian relationship. This was found in the anthocyan coloration of the stem. Harland (3), who has done much work in the West Indies on the genetics of the cowpea, states that in many varieties the region near the junction of petiole and stem is coloured dark red by the presence of anthocyan pigment in the epidermal cells, while in other varieties there is no trace of this coloration. He further states that the presence of this pigment in stem and leaf-stalk is dominant to its absence and is due to a single factor; all of his F_2 unpigmented families bred true to the unpigmented condition, and a cross between pigmented and unpigmented gave a ratio of three pigmented to one unpigmented in the second generation. The pigmentation is a definite and easily visible character,

and furthermore it has the advantage of being observable in the seedling stage, generally, as noticed by the writers, in the axil of the first pair of true leaves, and invariably by the time the second pair of true leaves has appeared. These results were verified in the present work by the observation of several thousand plants.

The parental types chosen for the work in hand were (*a*) a variety of bunched, determinate habit, mauve flowers and carrying the axillary pigmentation, which will be referred to as Axil Spot type; and (*b*) a variety of indeterminate habit, white flowers, and no pigmentation, which will be called Albino type.

Technique.

The parental types were grown under uniform conditions in one field plot; 11 Albino plants and 46 Axil Spot plants were cultivated. In all 14 crosses were made, 9 with the axil spot as female parent and 5 with the spot plant as male parent. The flowers were taken at random, and on the evening before the day on which the flowers would normally open those intended for female parents were castrated, the stamens being removed and counted and the flowers tied up with wool. The following morning they were untied and pollinated by pollen from still unopened flowers (fertilization normally occurring before opening) after which they were re-tied and marked by a piece of wool tied round the base of the flower. The pods, when mature, were collected and the seed sown on ridges, the distance between the ridges being 2 ft., and between the plants 18 in. Along with them was sown a number of selfed seed from the unpigmented parent for the purpose of comparison.

The F_1 seedlings were found to carry the character for pigment, and developed into plants with a determinate habit and mauve flowers like the spot parent. They were grown in the dry season and under irrigation conditions, and records were kept of irrigation, rainfall, temperature, and humidity. These will be found in Table II and Figs. 6 and 7. Growth was good in spite of an unevenness of germination, and the only trouble experienced was a slight attack of mosaic disease carried, probably, by a Chrysomelid beetle (*Diabrotica* sp.). Against this, two applications were made of a mixture composed of one part of magnesium arsenate to twenty-five parts of cassava flour, which proved effective in controlling the beetle attack and at the same time showed no harmful effect on the plant tissue, contrary to opinions expressed that leguminous plants cannot withstand arsenical insecticides.

The first flowers appeared on February 29, on which day five flowers, from three plants, were selfed. The number of flowers increased rapidly and up to March 11 inclusive all the flowers appearing were selfed. The method employed was to examine the plants in the evening and to tie up

with wool the corollas of all flowers which would normally open on the following day. After tying, an ordinary cardboard label was securely attached to the base of the flower, and on it was written the date of opening, i. e. the day after the actual tying up. Labels of different colours—red, green, yellow—were used on successive days in order later to facilitate the picking of the ripe pods. The following morning the plants were again examined to see that no flowers had been missed and to ensure that the flowers tied on the previous evening were still fastened. This was done between 6.30 a.m. and 7.30 a.m. before the flowers opened and before the Carpenter bee normally made its appearance; at any rate it was not seen.

This daily selfing was carried on from February 28 until April 4, a period of 36 days, and until March 11 all the flowers produced were tied up; 55 plants were then in flower, and on that day 267 flowers were selfed.

At this stage it was decided to discontinue the selfing of all the flowers produced, and merely to tie up sufficient on each day to ensure an F_2 population large enough for analysis. The pod of the cowpea contains an average of 10 seeds, and it was decided that 100 flowers selfed per day would yield a sufficient number of plants in the F_2 for accurate work. The main consideration in deciding this change lay in the work involved. The authors were reluctant to risk the possibility of mistakes through careless work by the employment of native assistants in the selfing of flowers and the collection of pods, and it had become impossible for them to cope unaided with the work of selfing of all flowers, particularly when the first-formed pods became ripe.

The amended system was that in the evening approximately 100 flowers were selected at random throughout the population and selfed. Next morning before opening, these flowers were examined to see that the tying wool and label were secure, and all other flowers not so tied up were pulled off. This procedure (1) reduced the chances of tied flowers being shed by reducing the drain on the plant, (2) facilitated harvesting of the selected pods. Incidentally this procedure would probably lengthen the flowering period of the plants. The system was continued throughout the rest of the experiment.

Throughout the whole experiment, in addition to the above work, a complete record was kept, for each plant of (*a*) the number of flowers tied and labelled per day, (*b*) the number of flowers pulled off per day, (*c*) the number of flowers shed per day. (This was done by collecting the labels which had fallen. Shedding was observed to take place two days after fertilization—only very occasionally were pods found to be shed at a later age. It was also observed that rarely were there more than three or four pods set on any one 'head'; any further flowers produced here were usually shed. There seemed to be a limited amount of nutrient supply

going to each flower-head, and when that limit was reached no more pods would be set.) (*d*) The number of pods harvested per day: (*e*) from the above records, by addition, was found the total number of flowers produced by each plant per day. These data gave a flowering curve for the whole population (Fig. 3), (*f*) the date of first flowering and last flowering: from these records it is seen that under the existing conditions the Cowpea has a definite flowering period, followed by a more or less definite non-flowering period; this may again be followed by a second, and even a third, flowering period. The frequency curves in Fig. 4 show that the first flowering period has a mean of about 23 days and the non-flowering period of about 12 days. The period from pollination of flower to mature pod corresponds in length to this non-flowering period, being 12 to 15 days. As might be expected, flowering begins again when the first flush of pods has been fully developed, as by that time the drain on the resources of the plant-body has greatly lessened. Similar results were found by Darbishire (2) working with *Pisum* sp. The method of harvesting was to collect the ripe pods from each day's flowering in separate bags, and at the same time to record the number produced by each plant. Thus at this stage, as explained above, we have a complete history of each individual plant. Observations on the flower production of the population were carried on until April 22, i. e. for eighteen days after tying up had ceased, so that it would be possible to say with certainty in what condition of flower production any plant was on April 4. The flowering and non-flowering periods have been extracted from the records and incorporated into Table VI.

The F_2 Generation.

Seed beds were prepared on plots where Cowpea had not previously been grown for a considerable time. To ensure that no 'foreign' Cowpea seedlings should be present the beds were watered some time before sowing and all seedlings which came up (and which were chiefly of *Ricinus*) were removed. Further, the tops of the beds were covered with a substantial layer of soil and pulverized manure which had been passed through a fine sieve.

The pods were shelled and the seed planted in rows (further to reduce the possibility of the inclusion in the final count of 'foreign' seedlings). The rows were two inches apart, with two inches between the seeds in the rows, and each bed was labelled with the date of selfing of the parents.

The germination of the seeds and growth of the seedlings were alike satisfactory. No plant was examined and finally classified as Axil Spot or Albino until the second pair of true leaves had appeared, and all precautions were taken to ensure accuracy of classification. In cases where

the spotting was faint (and considerable variation in intensity of spotting was noticed) the seedling was washed and carefully examined.

The ratios obtained are given in the accompanying table (Table I) and are plotted in Fig. 1.

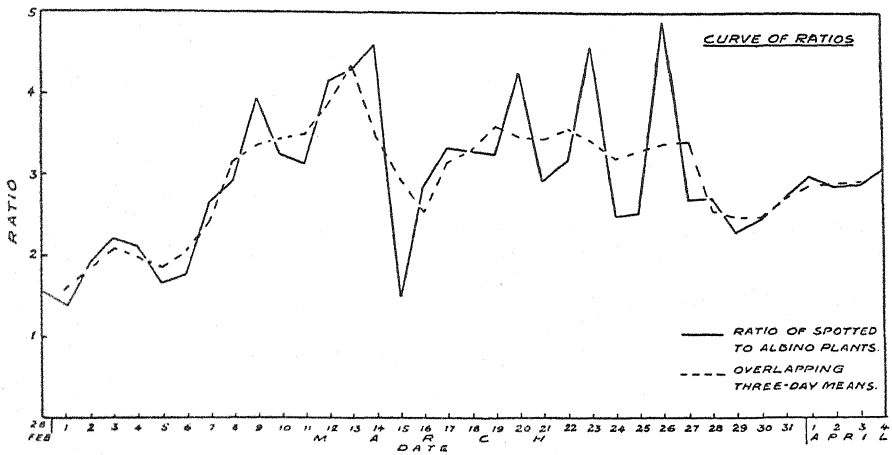


Fig. 1. *Vigna sinensis.* Curves showing the ratio of spotted to albino plants from February–April.

Analysis of the Results.

The first thing that claims our attention is the remarkable way in which the ratios vary from day to day. A range of 1·5 to 4·5 in populations which may be as large as 950 plants, and which average 450 over the whole 36 days is surely due to something more than chance variation. Moreover, the way in which the ratios steadily increase from 1·5 to 4·5, dip and then rise again, augurs something of significance. Compared with this, the fact that the total ratio of over 16,000 plants varies significantly from 3 : 1 is of secondary consideration, for it is obvious that if this daily variation be significant the final ratio will depend on the number of observations made, provided that these are sufficient to be statistically significant. It should be noted that after the first peak had been reached, the ratios, while varying, remain somewhere in the neighbourhood of 3 : 1, so that a ratio somewhere in that region will be obtained over a considerable range of time.

Looking at the table it will be seen that the ratios start in the neighbourhood of 1·5 : 1, i. e. in the first-formed flowers there is a strong tendency to produce recessive forms in undue numbers. As the plant increases in age the later-produced flowers produce relatively more and more dominant forms until by the 15th day the ratio of dominant to recessive is 4·58 : 1, i. e. is as far above the expected 3 : 1 as the ratios produced by the first flowers were below it. Here the ratio drops suddenly to its original value

of 1·5, and it is significant that this period of 15 days is that taken by the plant to mature its pods. The plants which first started flowering have

TABLE I.

Date.	Spot Plants.	Albino Plants.	Ratio Spot : Albino.	Overlapping. 3-day means.
Feb. 28	38	25	1·52	
Mar. 1	79	57	1·38	1·60
2	120	63	1·90	1·83
3	247	112	2·20	2·07
4	348	166	2·10	1·99
5	349	210	1·66	1·84
6	462	262	1·76	2·02
7	484	184	2·63	2·43
8	625	213	2·91	3·16
9	283	72	3·93	3·36
10	643	199	3·23	3·42
11	706	227	3·11	3·49
12	165	40	4·12	3·84
13	210	49	4·28	4·33
14	486	106	4·58	3·45
15	189	127	1·49	2·96
16	311	111	2·80	2·53
17	353	107	3·30	3·12
18	425	130	3·27	3·27
19	433	134	3·23	3·57
20	337	80	4·21	3·44
21	369	128	2·88	3·41
22	368	117	3·15	3·52
23	104	23	4·52	3·38
24	170	69	2·46	3·16
25	323	129	2·50	3·28
26	346	71	4·88	3·34
27	335	127	2·64	3·39
28	430	162	2·65	2·52
29	330	146	2·26	2·45
30	355	146	2·43	2·45
31	353	132	2·67	2·68
Apr. 1	371	126	2·94	2·81
2	402	143	2·81	2.86
3	400	142	2·82	2·89
4	276	91	3·03	
	12,225	4,426		
	16,651			

Total ratio $= \frac{12,225}{4,426} = 2{\cdot}76 : 1{\cdot}00$.

Expection from 16,651 plants on 3 : 1 basis is 12,489 spot and 4,163 albino.

Deviation of result from 3 : 1 ratio $= \pm 263$.

$\sigma = \sqrt{npq} = \sqrt{\frac{16,651 \times 3 \times 1}{4 \times 4}} = \frac{223{\cdot}5}{4} = 55{\cdot}9$.

Deviation is greater than 3σ and is $\therefore$ significant.

by this time reached their maximum and are ceasing to flower while the later plants are beginning to flower and are producing an excess of recessive forms (compare the flowering curve, Fig. 3). From this point the

ratio again increases, though somewhat erratically, since the population is now less uniform with regard to flowering age, and a second peak is reached

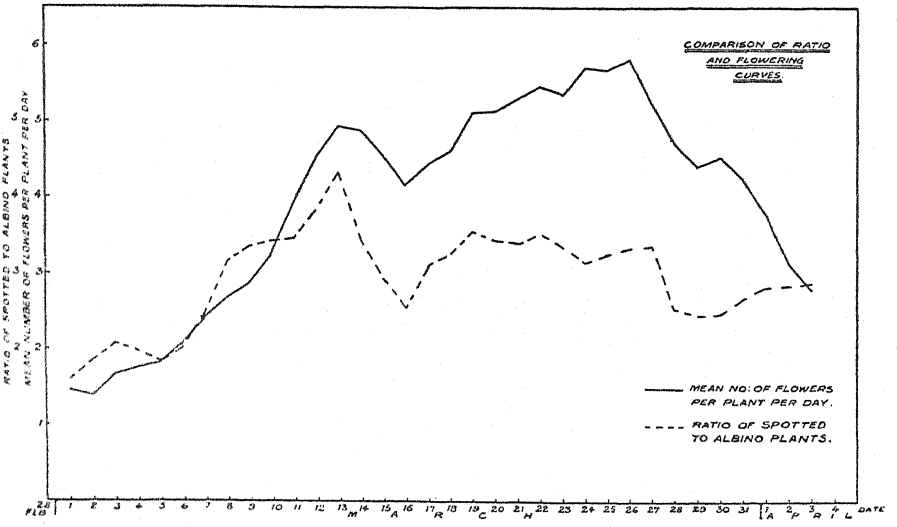


FIG. 2. *Vigna sinensis.* Curves showing mean number of flowers per plant per day and ratio of spotted to albino flowers.

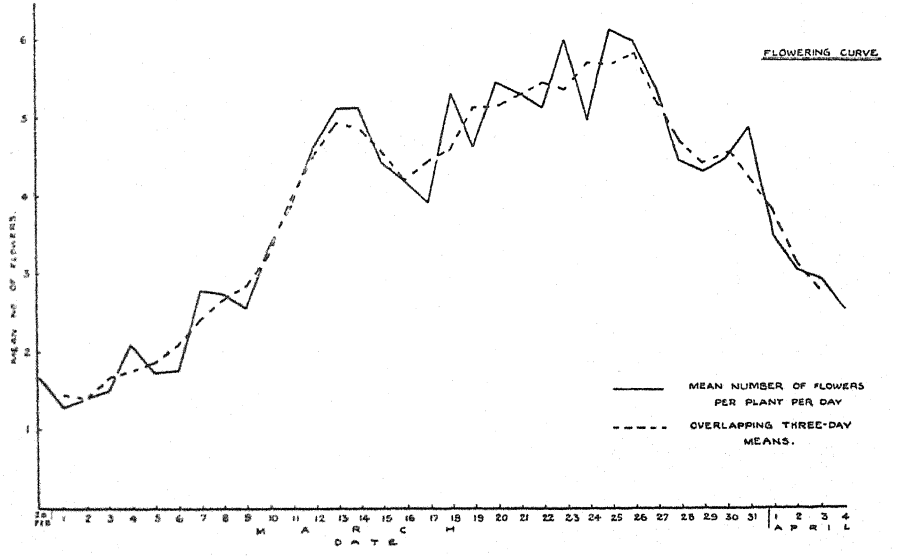


Fig. 3. *Vigna sinensis.* Flowering curves for February–April.

on March 26 or 14 days after the first peak. This figure is again significant, as denoting that the second maximum of flowering has been reached. There is now another drop, though not so great as before, and a suggestion of the commencement of another upward grade. The second peak is

slightly higher than the first, but is in the same neighbourhood of 4·5–5·0. It remains to find an explanation of this periodicity, although some connexion with the flowering period of the plant is already hinted at.

It is conceivable that the changes necessary in the mechanism of cell division to affect the segregation ratio should be the result of some change

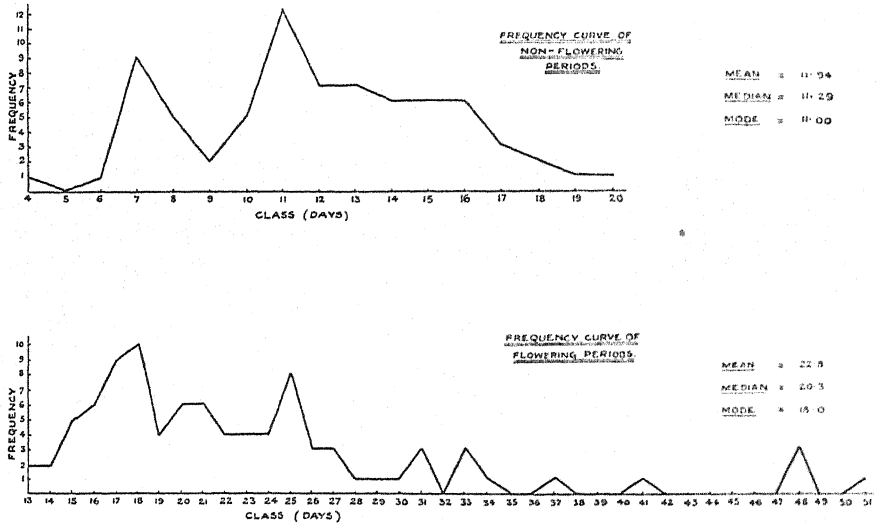


FIG. 4. *Vigna sinensis.* Frequency curves of non-flowering and flowering periods.

in the environment in which the plant is growing, or that they should result from natural changes during the ontogeny of the organism, unaffected by the external environment. Cases of the former process are uncommon, although there are cases known in which a sudden shock, produced by change of temperature or humidity, has resulted in the production of new forms, and it has been found that the dominance of abnormal abdomen in *Drosophila* is dependent upon a high moisture content in the atmosphere. It is, therefore, necessary first to examine the environment under which these plants were grown to see if an explanation for the variations can be found there. If this inquiry yields no result we shall be in a position to consider the question of some change in the plants themselves.

The Environment.

Soil. The plants were grown on a small plot on ridges 2 feet apart, and with 18 inches between successive plants. There were four such ridges, with 111 plants, an average of 25 plants per ridge. It is unlikely that any very great soil differences should occur in so small a space, and this is supported by the appearance of the plants themselves. Rates of germination were variable, but once grown the plants were very uniform in size. Before sowing, the whole plot was hoed and treated to a dressing of pul-

verized manure, and further treatment, which consisted only of irrigation, was uniform over the whole plot. The water supply was controlled and evenly distributed, and every effort was made to keep conditions as uniform as possible. The dates of irrigation are shown on Table II.

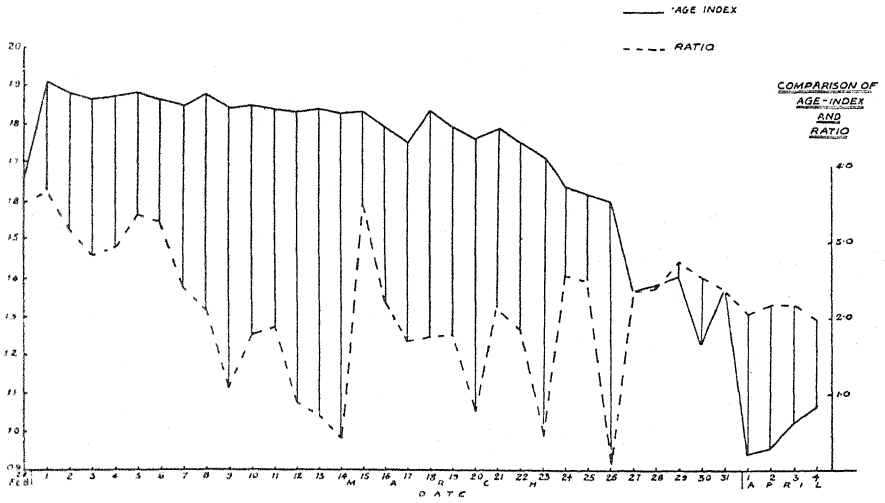


FIG 5. *Vigna sinensis.* Curves showing age-index and ratio.

Temperature.

An examination of Fig. 6 will show the extremely narrow range of temperature variation during the period of the experiment. The mean temperature shows a maximum variation of only 7°, while the greatest extremes (in the minimum temperatures) are 60° and 72° F.—a range of 12°. Overlapping three-day means would bring the two curves (maximum and minimum temperature) for all practical purposes constant and parallel in trend.

Humidity.

The plants were grown during the dry season and variation in humidity was not great (Fig. 7). The greatest humidity is found on the twelfth, on which day rain fell. It is coincidental that this rise in humidity should precede by a few days the drop in ratio, but no such correspondence is found at the time of the second drop in the ratios, and the humidity curve is certainly not periodic, as is that of the ratios.

Rainfall is given in Table II. There was very little rain during the experiment, and such as occurred shows no correspondence in time with the movements of the ratios.

It may be concluded from the above records that, as a factor responsible for such changes as have been observed, variation in the environment may be

neglected. We are, therefore, led to seek the cause of such aberration in the growth and structure of the plant itself.

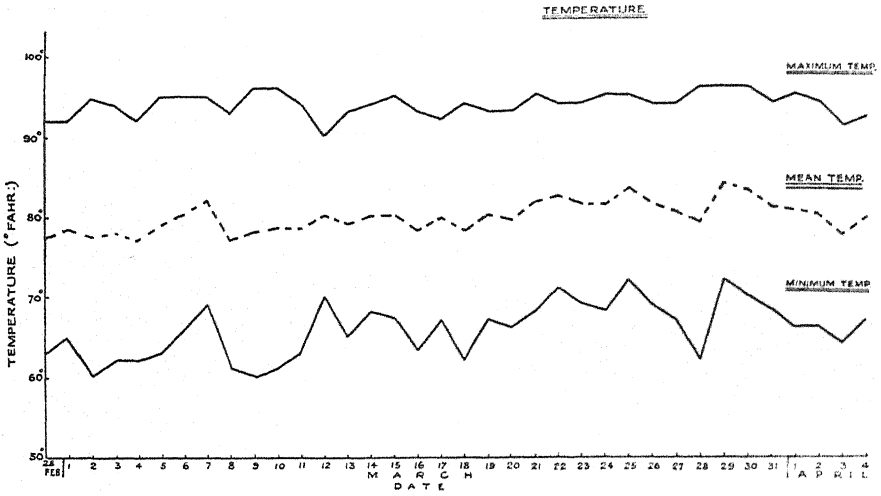


FIG. 6. Meteorological data showing maximum temperature, mean temperature, and minimum temperature for period February–April.

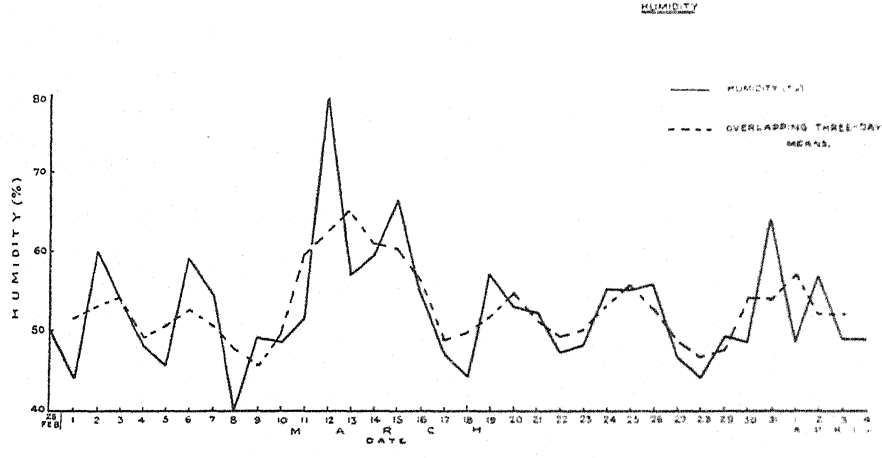


FIG. 7. Humidity data.

Influence of Parental Age.

If one compares the flowering curve (mean number of flowers per plant per day) with the curve obtained by plotting the ratios, using three-day means in each case (Fig. 2), one is struck by the general similarity of movement. Out of 33 observations there plotted, 21 show the curves moving in the same direction, while, out of the 12 cases in which direction of motion is different, 7 occur in the first four and last days, leaving in the

main body of the curve 20 cases of agreement to 5 of discrepancies. It is further seen that the first peak occurs on the same day (March 13) in the two curves, the first trough on the same day (March 16), and that whereas in the flowering curve the final drop commences on March 26, in the ratio curve it commences only one day later. The curves are particularly faithful up to March 16 (disregarding the natural fluctuations during the first few days), and these days are probably those from which interpretations can be made with most accuracy as far as the flowering curve is concerned, as will be seen later when it is analysed.

TABLE II.

Environment.

Date.	Rainfall.	General Weather.	Irrigation.	Other Treatment.
Feb. 28	—	Bright		
Mar. 1	—	,,		
2	—	,,		Dusted with magm. arsenate against *Diabrotica*
3	—	,,		
4	—	,,	1st irrigation	
5	—	,,		
6	—	,,		
7	—	,,		
8	—	,,		
9	—	,,		
10	—	,,		
11	—	,,		
12	0·19 in.	Fairly cloudy	2nd irrigation	
13	—	Bright		
14	—	,,		
15	—	,,	3rd irrigation	Dusted with magm. arsenate against *Diabrotica*
16	—	,,		
17	0·03 in.	Fair		
18	—	,,		
19	—	Bright		
20	—	,,		
21	—	,,		
22	—	,,		
23	—	,,	4th irrigation	
24	—	,,		
25	—	,,		
26	—	,,		
27	—	,,		
28	—	,,		
29	—	,,	5th irrigation	
30	—	,,		
31	0·06 in.	Cloudy		
Apr. 1	—	Bright		
2	0·22 in.	Moderate cloudiness		
3	—	Bright	6th irrigation	
4	—	,,		

This general agreement suggests a possible connexion between ratio and intensity of flowering on any one day, and since in the normal plant intensity of flowering and age of plant are related, up to a point, by a

simple curve, a connexion between segregation ratio and parental age is indicated.

The work of Zederbauer on the influence of parental age has already been dealt with, but it is hardly possible to draw comparison with his work; his consideration of age was made in the original parent and apparently no account was taken of the age of flowers in the F_1 generation, whereas the present work is concerned with the age of the F_1 plants, the original cross having been made with flowers taken at random on the parent plants.

Estimation of Parental Age.

It is usual in calculating the age of a plant to reckon from the date of germination. In the present work no idea of the possible connexion between age and ratio was originally entertained, Dr. Harland's chief aim in devising the experiment being to establish a relationship between variation in ratio and variation in environmental conditions, and no records of germination were kept. This information being unavailable one might start to calculate from the day of sowing, but this involves an assumption of uniformity of germination which in this case is certainly not justified. A glance through the records will show plants which have been omitted altogether; these germinated so late that they were only beginning to flower when the others had almost finished. Further evidence is obtained from the flowering curve (Fig. 3). Examination of the record of any one plant will show that its rate of flowering (measured by number of flowers) increases to a maximum and then falls off either to zero or to a condition of occasional spasmodic production. This non-flowering period lasts on the average about 12 days, after which flowering recommences, again increases to a maximum and again falls off. An ideal flowering curve should therefore be bimodal; the curve obtained (Fig. 3) from the combined records of 91 plants is bimodal, but the dip between the two peaks is nothing so great as expected from theory. It is found, however, that it is necessary to take overlapping 18-day means before the dip is eliminated and that the distance between the two peaks is 13 days (March 13–26), which is very near the mean non-flowering period for the whole population. It is, therefore, concluded that the dip is significant and that the curve is truly bimodal. The departure from theory in this respect would then be accounted for by 'overlapping' caused by commencement of flowering at different dates. It is seen from Table VI that commencement of flowering of individual plants varied from February 28 to March 21; the flowering curve obtained is therefore a composite one, combining the records of 91 plants which had appreciably different commencing dates. On March 13, when the flower production of the early plants was falling away, that of the later plants was still increasing, the result being that the theoretical condition of

'no-flowering' was never reached. It follows that the most reliable part of the curve, for deductive and comparative purposes is the first 15 days, before overlapping seriously commenced.

Such overlapping may arise from either of two causes or from a combination of the two. These causes are (1) uniform germination, followed by appreciable differences in rate of growth, caused by heterogeneity in composition of the plants, or by differences in soil or atmospheric conditions, (2) uniform composition of plants and conditions of growth but unequal germination. Now we know already that germination was unequal from the exceptional cases already cited; it remains to ascertain, if possible, the conditions prevailing among the plants from which those records were taken.

On Fig. 4 will be found frequency curves of first-flowering and non-flowering periods of the plants under examination. While the curve of flowering periods is far from ideal, there being several cases of exceptionally long flowering, the mode, mean and median are fairly close together. That of the non-flowering periods, however, shows a uniformity which is surprising considering the small number of observations. In spite of the size of the class '7-days' the mode, mean and median come all in the same class, and with more observations classes 8, 9, and 10 would probably be larger (being near the mean) and the curve would come near to the normal form. These records are considered strong evidence of the uniformity of composition and treatment of the population, backed up as they are by personal observation and supervision throughout the experiment. It has already been shown that the effect of the environment is negligible.

It would seem fairly safe, then, to ascribe the overlapping to unequal germination, in which case it is quite impossible to use the date of sowing in any determination of parental age. It is proposed here to use as a basis for this calculation the date of first flowering, and to count age from there. There is little difference, for what is required is an index for purposes of comparison, and not an absolute figure.

The determination of this index will now be explained. The bed of F_2 plants corresponding to any one day's flowering is raised from seed from many different plants, of different 'ages'. Moreover, if segregation ratio is affected by parental age, the total ratio for the day, being a combination of the ratios from different plants of different ages, will be affected by the proportion in which those plants contribute. To take a very simple case, imagine that two plants only are contributing pods to the F_2 population. One of these plants is segregating in a 5 : 1 ratio, the other in a 2 : 1. The production of equal numbers of seeds by these two plants would give a total ratio of 3 : 1, whereas twelve seeds from the first plant and three from the second would give a ratio of 12 : 3 or 4 : 1. All we know is the final ratio, and since this is obviously affected by percentage contribution,

and we wish to compare ratio with age, we must bring percentage contribution into our calculation of age index. We have, therefore, to take into account in our determination of the 'average age' of any seed bed both the 'age' of the parent plants and the number of pods contributed by each plant.

The basis of these calculations is found in Table VI which gives the individual flowering record of every plant. This information is abstracted from the records and arranged as shown. For each plant flowering periods are left blank, while non-flowering days are outlined in black, the crosses indicating the days on which flowering began and ceased. The records of flowering were continued for eighteen days after the last day of tying up, so that the condition of any plant could be judged at that time. Counting the first cross as '1' the succeeding days are numbered off up to April 4. No difference is made in the numbering when a plant temporarily ceases to flower; it would be manifestly unfair to stop the counting here, and to carry on at the same figure, or to start again, when the second flowering began; in fact, in those cases where flowering did not cease entirely such a course would be impossible. This is all self-adjusted in the next stage. For each day a list is made of the 'age' of each plant by reading vertically down the desired column of the Table. Then from the records the number of pods set by each plant is ascertained, and this number is multiplied by the 'age' of the plant. The products so obtained are summed, and divided by (1) the number of plants concerned, i.e. setting pods on that day and (2) the total number of pods produced on that day. The resultant quotient is a number, as will be seen from the expression:—

$$\text{Age Index} = \frac{(\text{No. of Pods} \times \text{'age' of Plant})}{\text{Total Pods} \times \text{Total Plants}}$$

The adjustment for non-flowering periods will now be clear. When a plant is not flowering it is setting no pods, its product (Pods × Age) will be zero, and it will disappear from the final records. For purposes of illustration the calculation of the Index for March 23 will be given in full (Table III).

TABLE III.

Calculation of Age Index for March 23.

No. of Plant.	'Age' of Plant (from table).	Pods Set (from records).	Product Age × Pods.	No. of Plant.	'Age' of Plant (from table).	Pods Set (from records).	Product Age × Pods.
8	10	1	10	60	10	1	10
9	11	2	22	61	5	—	—
10	9	2	18	62	3	—	—
11	6	1	6	67	20	—	—
12	18	1	18	68	22	—	—
13	9	—	—	70	20	—	—
14	20	1	20	72	20	—	—
15	21	—	—	73	16	1	16
16	18	2	36	74	19	6	114
17	12	2	24	76	24	—	—
18	16	1	16	77	6	2	12
19	12	—	—	78	16	—	—
20	10	1	10	79	10	2	20
21	22	1	22	80	23	—	—
22	13	1	13	81	19	—	—
23	23	—	—	82	8	—	—
24	12	3	36	83	18	—	—
25	19	—	—	85	23	—	—
26	16	1	16	86	20	—	—
27	18	3	54	87	12	1	12
29	23	2	46	88	23	—	—
30	24	—	—	89	23	—	—
31	13	2	26	90	20	—	—
32	15	2	30	91	21	—	—
33	12	1	12	92	18	1	18
34	6	1	6	93	23	—	—
35	14	1	14	94	23	—	—
36	18	1	18	95	23	—	—
37	21	2	42	98	21	—	—
38	24	—	—	99	23	2	46
39	19	1	19	100	11	—	—
40	23	—	—	101	19	—	—
41	16	—	—	103	21	—	—
42	22	1	22	105	14	—	—
43	16	3	48	107	13	2	26
44	19	—	—	108	18	—	—
45	11	1	11	110	19	—	—
47	12	3	36	111	16	—	—
48	13	1	13	112	21	—	—
49	17	2	34	113	19	—	—
53	16	1	16	114	11	4	44
54	8	3	24	115	18	—	—
55	22	—	—	116	12	3	36
56	16	1	16	117	16	3	48
57	12	2	24	118	9	1	9
58	11	4	44	122	17	—	—
59	8	1	8			88	1,241

Σ Products = 1241. No. of plants concerned = 49.
No. of pods set = 88.

$$\therefore \text{Index} = \frac{1,241}{49 \times 88} = 0{\cdot}288.$$

This figure is calculated for each day, and the indices obtained are given in the following table (Table IV).

TABLE IV.

Age-Indices.

Date.	Σ P.	Plants.	Pods.	Index.
Feb. 28	5	3	5	0·333
Mar. 1	20	13	17	0·091
2	45	16	23	0·122
3	95	20	35	0·135
4	184	28	53	0·124
5	239	33	59	0·123
6	375	35	78	0·137
7	672	40	113	0·149
8	709	51	115	0·121
9	851	47	117	0·155
10	1,050	51	138	0·149
11	1,280	55	152	0·153
12	556	48	70	0·165
13	600	53	73	0·155
14	711	54	80	0·165
15	688	54	76	0·160
16	584	47	62	0·200
17	692	44	65	0·242
18	933	62	94	0·160
19	872	54	80	0·202
20	925	48	83	0·232
21	1,142	58	96	0·205
22	1,142	54	87	0·243
23	1,241	49	88	0·288
24	1,175	39	83	0·362
25	1,165	42	73	0·378
26	1,269	44	73	0·395
27	1,120	29	63	0·613
28	1,617	30	86	0·627
29	1,672	32	88	0·594
30	1,648	27	79	0·773
31	1,503	36	67	0·623
Apr. 1	1,873	25	71	1·055
2	1,958	23	82	1·038
3	1,770	26	70	0·973
4	1,534	29	57	0·928

If we compare the indices in Table IV with the segregation ratios we shall find a close agreement in the direction of movement of the two sets of figures. This will be seen from the following table (Table V) where are arranged the indices and ratios, with differences between successive values.

TABLE V.

Comparison of Age Index and Ratio.

Date.	Index.	Index Difference.	Ratio Difference.	Ratio.
Feb. 28	0·333			1·52
Mar. 1	0·091	−0·242	−0·14	1·38
2	0·122	+0·031	+0·52	1·90
3	0·135	+0·013	+0·30	2·20
4	0·124	−0·011	−0·10	2·10
5	0·123	−0·001	−0·44	1·66
6	0·137	+0·014	+0·10	1·76
7	0·149	+0·012	+0·87	2·63
8	0·121	−0·028	+0·28	2·91
9	0·155	+0·034	+1·02	3·95
10	0·149	−0·006	−0·70	3·23
11	0·153	+0·004	−0·12	3·11
12	0·165	+0·012	+1·01	4·12
13	0·155	−0·010	+0·16	4·28
14	0·165	+0·010	+0·30	4·58
15	0·160	−0·005	−3·09	1·49
16	0·200	+0·040	+1·31	2·80
17	0·242	+0·042	+0·50	3·30
18	0·160	−0·082	−0·03	3·27
19	0·202	+0·042	−0·04	3·23
20	0·232	+0·030	+0·98	4·21
21	0·205	−0·027	−1·33	2·88
22	0·243	+0·038	+0·27	3·15
23	0·288	+0·045	+1·37	4·52
24	0·362	+0·074	−2·06	2·46
25	0·378	+0·012	+0·04	2·50
26	0·395	+0·017	+2·38	4·88
27	0·613	+0·218	−2·24	2·64
28	0·627	+0·014	+0·01	2·65
29	0·594	−0·033	−0·39	2·26
30	0·773	+0·179	+0·17	2·43
31	0·623	−0·150	+0·24	2·67
Apr. 1	1·055	+0·432	+0·27	2·94
2	1·038	−0·017	−0·13	2·81
3	0·973	−0·065	+0·01	2·82
4	0·928	−0·045	+0·21	3·03

It is seen that, with nine exceptions (on March 8, 11, 13, 19, 24, 27, 31, and on April 3 and 4) out of a total of thirty-six observations, the two series move in the same direction. Of the discrepancies the last five are also dates of discrepancy between ratio curve and flowering curve. Apart from these exceptions an increase in age index is accompanied by an increase in ratio, and a decrease by a decrease. The numerical values of the changes are not comparable, but this is hardly to be expected and, in any case, the most important thing is the direction of movement. This is also seen from the plotted results (Fig. 5).

We are, therefore, led to the conclusion that in some way the segregation ratio varies and that it varies directly, with parental age. This is

only in the nature of a preliminary inquiry, but sufficient has been shown to suggest further work, particularly into the more exact nature of the relationship.

It is worthy of note that, assuming the age index to be correct in direction of movement, the total of the discrepancies in the ratios is in a negative direction, that is, the ratios are lower than they should theoretically have been, indicating a greater number of albinos than expected on a 3 : 1 basis. While every possible care was taken in classification, it is possible that mistakes were made, and any such mistakes are bound to have been in that direction. With the technique used, it is possible to classify a spot plant as an albino if the spot developed very late, but quite impossible to classify an albino as spotted. The correction of any errors made, therefore, would tend only to eliminate the discrepancies present and to strengthen still further the agreement between age and ratio.

Conclusions.

The important point is established that variation in segregation ratio occurs during the flowering period of a heterozygous plant. At different stages of the flowering period different ratios will be obtained. even where conditions of growth are uniform, and random sampling would appear to be an unsafe method in the determination of segregation ratios.

The deviations here obtained also form a strong argument in favour of the construction of the theoretical population, in cases of suspected linkage, from observed results rather than from Mendelian expectation (Collins (1)). If, say, in the inheritance of two pairs of factors, a departure from 9 : 3 : 3 : 1 is obtained, the ratios of the factors considered separately should be examined before linkage is assumed.

If the inheritance is simple Mendelian each pair of factors should give a 3 : 1 ratio, whether there is linkage or not. If variation from 3 : 1 is obtained the departure from 9 : 3 : 3 : 1 should be re-examined before linkage is assumed.

The results here obtained further suggest that variation is connected with the age of the parent plant. With few exceptions the ratios obtained vary in the same direction as the index used. It is impossible from these figures to calculate the exact connexion, but it would probably be enlightening to repeat the experiment, keeping each plant entirely separate and individual.

Summary.

1. The F_1 of a monofactorial cross between varieties of *Vigna sinensis* was grown, and the seed set from each day's flowering kept separate. The seed was grown and the ratio of dominant and recessive forms calculated separately for each day.

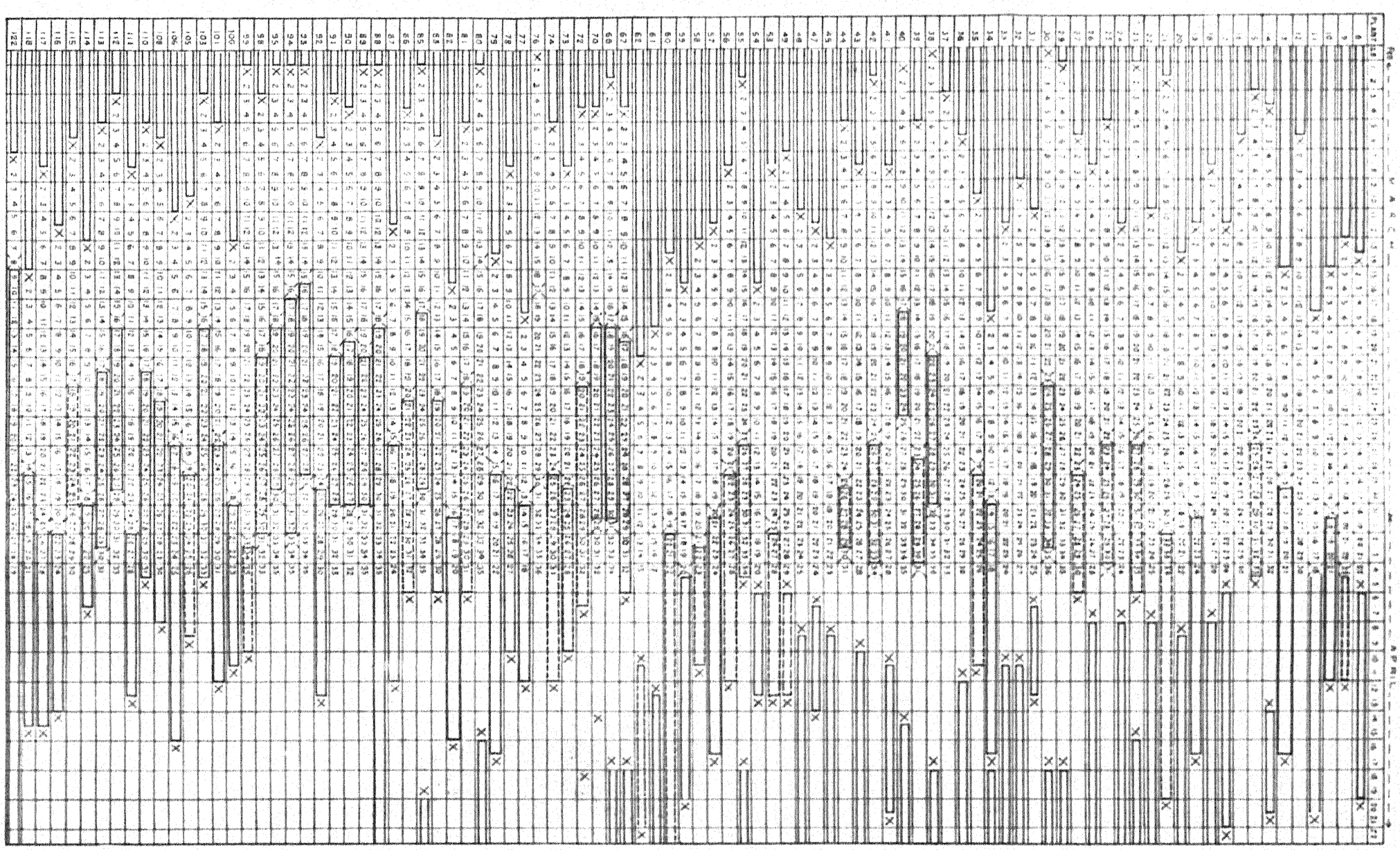


CHART OF FLOWERING RECORDS.

TABLE VI

THE DATE OF FIRST FLOWERING IS MARKED BY A CROSS; A SECOND CROSS INDICATES CESSATION OF FLOWERING, AND A THIRD, COMMENCEMENT OF SECOND CROP OF FLOWERS.

__ NON-FLOWERING PERIOD.

__ PERIOD OF VERY INFREQUENT FLOWER PRODUCTION; FOR ALL PRACTICAL PURPOSES, A NON-FLOWERING PERIOD.

THE FIGURES, COMMENCING AT DATE OF FIRST FLOWER, ARE USED AS A BASIS FOR THE DETERMINATION OF PARENTAL AGE (SEE TEXT)



2. It was found that the segregation ratio varied from day to day, such variation being periodic. It was shown that environmental conditions were not responsible for the variation. By the use of an index for purposes of comparison, some connexion with the average age of the parent generation was established, and it was shown that segregation ratio varied directly with parental age.

3. At the same time, flowering records of the plants were kept, and it was established that the Cowpea (*Vigna sinensis*) had definite flowering and non-flowering periods which were constant for the variety used.

Our thanks are due to Professor S. C. Harland for the suggestion of the experiment and of the technique employed.

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The Interaction of Factors in the Growth of Lemna.

IV. The Influence of Minute Quantities of Organic Matter upon Growth and Reproduction.

BY

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With two Figures in the Text.

I. INTRODUCTION.

IN 1914 Bottomley published the first of a series of papers on plant nutrition, in which he set out to demonstrate that inorganic substances alone were inadequate for the nutrition of the green plant (1). In subsequent papers (2, 3, 4, 5) Bottomley and Mockeridge showed that certain complex organic substances, when partially broken down by the action of nitrogen-fixing bacteria, stimulated the growth of *Lemna* and other water-plants to a marked degree; to the substances which stimulated growth Bottomley gave the name 'auximones'.

The conditions under which these experiments were carried out were not altogether satisfactory, and the necessity of organic substances for growth has since been questioned by a number of writers (6, 7, 8, 9). Clark and Roller (6) demonstrated that *Lemna* would grow as well in a dilute and suitably balanced inorganic medium as in a medium containing soil, and they attributed the falling off in growth of Bottomley's control cultures to the unsuitability of Knop's and Detmer's solutions. In subsequent papers Clark published details of an inorganic medium in which he grew *Spirodela polyrhiza* (*Lemna major*) for long periods out of contact with any organic materials. There seems no doubt from Clark's results and from those of the present writer (14), that *Lemna* can grow and multiply for indefinite periods in a purely inorganic medium; this does not, however, preclude the possibility that small quantities of organic matter may increase the growth-rate. Wolfe (8) reviewing the auximone question, suggests

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that the feeble growth of Bottomley's controls was due to lack of suitable 'physiological balance' in the solutions he employed; and concludes that organic matter is not *essential* for the growth of the green plant.

Bottomley and his co-workers did, however, report very striking increases in the growth-rate of *Lemna*, when certain organic substances were added to the solution in which the colonies were growing, and the phenomenon seemed worthy of more critical investigation. Bottomley's results require repetition for the following reasons:

(1) The criterion of growth used in much of Bottomley's data, namely, the number of *plants* in the colony, has been shown to be unsatisfactory as a measure of growth.

(2) The external conditions of temperature and light under which the experiments were performed, were insufficiently controlled, and not fully described.

(3) *Lemna* has been shown by Clark (9) to be sensitive to changes in pH. Any changes in pH, due to the addition of organic extracts, were apparently neglected by Bottomley. The present writer has found that the addition of some extracts (e.g. a manure extract as described by Mockeridge (5)), does in fact alter the pH considerably.

(4) In many of Bottomley's and Mockeridge's experiments there is insufficient information as to how much organic matter was added.

(5) In all the experiments described, the precautions taken to prevent bacterial infection and the entrance of dust were unsatisfactory.

(6) In the experiments described, the solutions were changed only twice a week. Clark has shown (9) that the growth-rate is strikingly modified unless the solutions are changed very frequently.

(7) The results published by Mockeridge and Bottomley were not subjected to statistical analysis, and the experimental error is often so great that the significance of their results is uncertain.[1]

Moreover, the studies of previous workers needs elaboration in two respects. Evidence has not been given as to the effect of the growth-promoting substance on the various growth processes, and secondly, there is insufficient evidence from the data to hand as to whether the action of the organic matter is catalytic in nature, or whether it increases proportionally to the amount present. If the action is of a catalytic nature, much smaller amounts than Bottomley added (3, p. 487) should be effective. This would appear to be one of the most urgent problems connected with the assimilation of organic matter by green plants.

It seemed advisable, therefore, to investigate the question of 'auximones' afresh, and for this purpose the technique for the micro-analysis of growth evolved by the writer and others (12, 13, 14) seemed suitable; since the

[1] For instance, the significance of the results with fresh manure (5, p. 437, vii), is doubtful.

experiments could be carried out under conditions far more carefully controlled than heretofore. The chemical nature of the substances concerned has received the attention of Bottomley and Mockeridge, and is not considered here.

II. Methods.

1. The source of organic material was fresh horse dung. This was chosen because of its obvious agricultural significance.

Extraction. About 200 grm. of fresh horse dung were placed in 1 litre of 'glass-distilled' water and kept for two hours at 50° C. The thick extract was then filtered and run into flasks, each containing 100 c.c. which were then tightly plugged with cotton wool.

Some of these flasks were sterilized by autoclaving for one hour at 120° C. on two successive days. The rest were 'pasteurised' by being subjected to a temperature of 55° C. for three hours on six successive days. The flasks were stored in a refrigerator at 0° C. until required for use.

2. *Analysis.* The extract of dung so obtained was of unknown concentration and composition. The concentration was found by evaporating three samples of 50 c.c. each to dryness *in vacuo*, and weighing the residues. Samples of the solution were analysed for nitrogen by the micro-Kjeldahl method.[1] The results of these analyses are set out in Table I.

Table I.

Sample 50 c.c.	Wt. of Dry Matter.	Sample 100 c.c.	Wt. Nitrogen.
1	0·2070 grm.	1	0·053 grm.
2	0·1988	2	0·057
3	0·2017	3	0·058
	0·2028 (mean)		0·056 (mean)

Solid matter per c.c. 0·00406 grm.
Nitrogen per c.c. 0·00056 grm.

3. *Solutions.* The medium used was the inorganic solution recommended by Clark (9), which has been employed for all the experiments on *Lemna* in this laboratory. This solution contains 1·21 grm. solid matter per litre, of which 0·11 grm. is the nitrogen present in KNO_3. A pure clone of *Lemna* was used which had been growing for six months at a light intensity of 500 foot-candles, and at about 25° C. exclusively in Clark's solution. The inorganic medium was made up with great care from 'glass-distilled' water, and was stored in litre flasks. It was sterilized in the autoclave before the addition of the organic extracts.

[1] I have to record my thanks to Miss E. H. M. Farries for undertaking these analyses.

For the actual experiments, the solutions given in Table II were prepared:

TABLE II.

Solution.		Extract added (c.c. per litre).	Dry Weight of Extract added (grm. per lit.).	Weight N added (grm. per lit.).
A	Standard solution	+ 0·00 —	0·00	0·003 (as KNO_3)
B	„ „	+ 5·00 reduced to ash	0·0075	—
C	„ „	+ 0·05 pasteurised	0·0002	0·00003
D	„ „	+ 0·50 „	0·002	0·0003
E	„ „	+ 5·00 „	0·02	0·003
F	„ „	+ 5·00 autoclaved	0·02	0·003

The ash was obtained by heating 5 c.c. of the solution in a platinum crucible until the residue was white.

The addition of nitrogen as KNO_3 to solution A was probably quite superfluous, but it shows that any effect due to the addition of organic matter is not merely the result of an extra nitrogen supply.

The addition of the dung extract in solutions B, E, and F changed the pH from 4·8 to about 5·0. The pH was restored to 4·8 by the addition of N/200 H_2SO_4.

When the extract was evaporated the dry material was found to contain approximately 36 per cent. of ash.

The solutions were stored in stoppered litre flasks in a refrigerator at 0° C. until required for use.

4. *Experimental Procedure.* To investigate the nutritive effect of these six solutions, colonies of *Lemna minor* were grown in them under constant conditions of light and temperature and in constantly circulating air. The colonies were in sterile plugged conical flasks of 200 c.c. capacity. Three flasks of each solution were employed, so that there were eighteen flasks in all. The flasks were arranged at random in rectangular formation in one of the chambers of the apparatus described by Bolas and Henderson (11). This chamber rotated slowly under 1,500 watt Mazda lamps, giving a continuous illumination of 550 foot-candles, in a room at a temperature of approximately 25° C.; the temperature being controlled by a thermostat to within 3° C. Every forty-eight hours the colonies were transferred to a fresh set of sterile flasks. This precaution, together with the fact that the flasks were plugged with cotton wool, minimized any risk of infection or introduction of foreign matter. The experiment lasted eleven days. At the beginning, and after five, nine, and eleven days, the plant number, the frond number, the dry weight, the area, and the carbon content were found by the methods described in previous papers (12, 13, 14).[1] There was obtained, therefore, full data of the growth of the colonies in the six different solutions. The results of this experiment are set out in Table III.

[1] For analysis, two samples, each of twenty-five fronds, were taken at random from each experimental set.

TABLE III.

Solution.	0 Days. Fronds.	0 Days. Frond Weight.	0 Days. Frond Area.	5 Days. Fronds.	5 Days. Frond Weight.	5 Days. Frond Area.	9 Days. Fronds.	9 Days. Frond. Area.	11 Days. Fronds.	11 Days. Frond Weight.	11 Days. Frond Area.
A 1	100	0·035	0·021	194	0·034	0·020	314	0·022	482	0·034	0·021
A 2	100			196	0·037	0·021	321		420	0·037	0·021
A 3	100			179			310		412		
Mean	100	—	—	190	0·036	0·021	315	—	438	0·035	0·021
B 1	100	0·035	0·021	195	0·036	0·023	—		459	0·033	0·020
B 2	100			180	0·035	0·019	283	0·022	384	0·033	0·020
B 3	100			197			309		406		
Mean	100	—	—	191	0·036	0·021	296	—	416	0·033	0·020
C 1	100	0·035	0·021	222	0·037		395	0·022	546	0·034	0·023
C 2	100			205	0·035		395		560	0·033	0·021
C 3	100			—			—		—		
Mean	100	—	—	214	0·036	—	394	—	553	0·034	0 022
D 1	100	0·035	0·021	245	0·028	0·025	445	0·027	612	0·035	0·027
D 2	100			230	0·032	0·026	440		625	0·035	0·026
D 3	100			230			440		636		
Mean	100		—	235	0·030	0·026	442	—	624	0·035	0·027
E 1	100	0·035	0·021	220	0·037	0·027	400	0·028	672	0·038	0·028
E 2	100			240	0·036	0·027	432		630	0·036	0·028
E 3	100			233			430		656		
Mean	100		—	231	0·037	0·027	421	—	653	0·037	0·028
F 1	100	0·035	0·021	238	0·037	0·026	400	0·030	608	0·038	0·029
F 2	100			242	0·037	0·029	435		587	0 034	0·026
F 3	100			250			459		690		
Mean	100		—	243	0·037	0·028	431	—	628	0·036	0·028

III. Experimental Data.

A glance at the Table shows that even after five days there is a marked difference in growth between the sets. This difference can only be due to the addition to some of the sets of minute amounts of organic matter. The frond weight, even at the end of the experiment, is the same in all the colonies, although the frond area is 33 per cent. higher in 'E' than in 'A'.

IV. Analysis of the Results.

Since the experiments at each concentration were carried out in triplicate, the significance to be attached to the values can be calculated. That the minute amounts of organic extract added had a significant influence upon the growth-rate is clearly demonstrated in Table IV. This table gives the values of 't' for the differences of the means, calculated by the elaborated 'Students' method described by Fisher (10).

Table IV.

Significance of Differences of Means of Values for Frond Number, on the Eleventh Day of the Experiment.

Solution.	A.	B.	C.	D.	E.	F.
Mean $\bar{x}$	438	416	553	624	653	628
$S(x-\bar{x})$	2940	2735	98	25	900	5101
(n^1+1)	3	3	2	3	3	3

For mean of A minus mean of B $t = 0{\cdot}71$, $n = 4$, $P = 0{\cdot}5$; not signif.
" " A " " C $t = 4{\cdot}70$, $n = 3$, $P = 0{\cdot}02$; signif.
" " C " " D $t = 13{\cdot}80$, $n = 3$, $P = 0{\cdot}01$; signif.
" " D " " E $t = 2{\cdot}33$, $n = 4$, $P = 0{\cdot}1$–$0{\cdot}05$; not signif.
" " E " " F $t = 0{\cdot}79$, $n = 4$, $P = 0{\cdot}5$–$0{\cdot}4$; not. signif.

From Table III it will be seen that the frond weight at the end of the experiment is about the same in all the colonies. The values of 't' for frond number given above will therefore hold for total dry weight. The frond area is markedly increased in the colonies containing organic extract: *a fortiori*, therefore, the sets which show significant differences of frond number, will show differences of even higher significance for total area.

It is clear from the above results that an addition of 0·2 parts of organic matter per million, to a solution containing already 1,210 parts per million of mineral matter, will significantly affect the growth of *Lemna*. The power of the extract is not impaired by autoclaving: the effect, then, is not due to an enzyme. The ash constituent of the extract does not increase the growth-rate, since the growth in solution B does not differ significantly from that in solution A. It has been suspected that the active

substance in 'growth-promoting' material is a nitrogenous compound. It is interesting to note in this connexion that normal Clark's solution contains 0·11 grm. nitrogen per litre, as KNO_3. Solution A contains an additional 0·003 grm. nitrogen per litre added as KNO_3; while solution C has

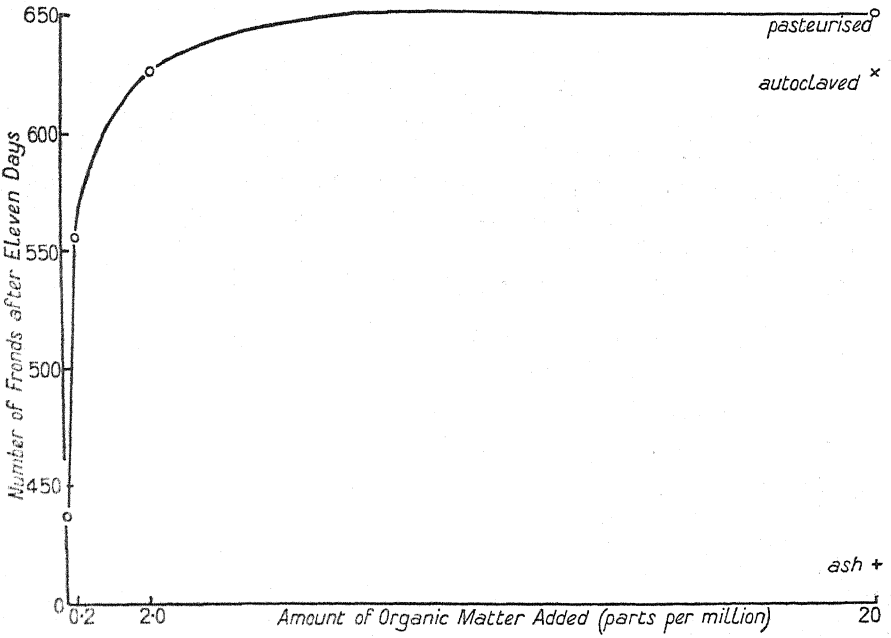


FIG. 1. Along the ordinates are measured the average number of fronds in the several experimental sets, after eleven days' growth. The abscissae represent the amounts of organic matter added, 0 to 20 parts per million.

additional nitrogen to only *one hundredth* of this amount but added as organic matter. The growth in solution C was, however, significantly greater than that in solution A.

The increase of the growth-rate must therefore be due to some 'catalytic' action of the organic matter, and not merely to a quantitative effect of the minute amounts of nitrogen added.

Further evidence as to the nature of the action is given in Table IV, and Fig. 1. It is clear that any addition of organic matter above a concentration of two parts, by weight, in a million produces no significant increase in the growth-rate.

It is of interest that the increase of growth is produced by quantities of organic matter, apparently much smaller than used by Bottomley so far as can be gathered from the data in his publications. The effect of the extract is apparent after about a week; Mockeridge, however, did not obtain significant differences with extracts of fresh manure until some time

after a fortnight's growth (5, p. 437). This may be due to the unsuitability of the inorganic medium she employed, or to the pH having been changed by the addition of the extract; though as she does not give any idea of the amount of material added, it is uncertain how strong her extract was. Indeed, at high concentrations of two thousand parts of organic matter per million, the writer found some inhibitory action upon growth; though the results were not very reliable owing to bacterial infection.

Mockeridge states (5, p. 436) that older manure is more effective. The results of a further experiment in which older manure was employed are given in Table V. It will be seen that the results do not agree with what Mockeridge found, since the increase in growth-rate is not significantly bigger than that found in the previous experiment.

TABLE V.

Experiment at 660 Foot-candles, and 25° C. Extract obtained from Older Well-rotted Manure.

Day.		0 Fronds.	Frond Weight.	Frond Area.	4 Fronds.	7 Fronds.	12 Fronds.	Frond Weight.	Frond Area.
Control	A 1	100			232	330	740		
	A 2	100			200	340	800		
	A 3	100			228	390	860		
Mean		100	0·040	0·019	220	353	800	0·040	0·021
1·9 parts	B 1	100			265	510	960		
per mill.	B 2	100			266	540	965		
	B 5	100			318	560	950		
Mean		100	0·040	0·019	283	537	958	0·039	0·026
1·9 parts	C 1	100			362	600	1035		
per mill.	C 2	100			340	560	1040		
Mean		100	0·040	0·019	351			0·041	0·030

It will be seen that essentially the same results were obtained as in the experiment previously described. The percentage increase of the frond area in the treated over the untreated populations is comparable with that in the previous experiment. The frond weight remains about the same, whether organic matter is added or not. The increase in growth-rate due to the addition of organic matter is not very different from the increase reported for the first experiment. The two experiments, however, are not strictly comparable, since the light intensities were not the same.

All the growth-curves (Fig. 2) approximate to exponentials though there is a slight diminution in 'r' after about eight days. This is possibly due to the fact that the flasks in which the colonies grew were tightly plugged with cotton wool, resulting in some shortage of CO_2.

V. SPECIFIC EFFECTS OF THE ORGANIC MATTER.

Further light can be thrown upon the nature of the action by a closer analysis of the results. In general, a striking increase in the growth-rate

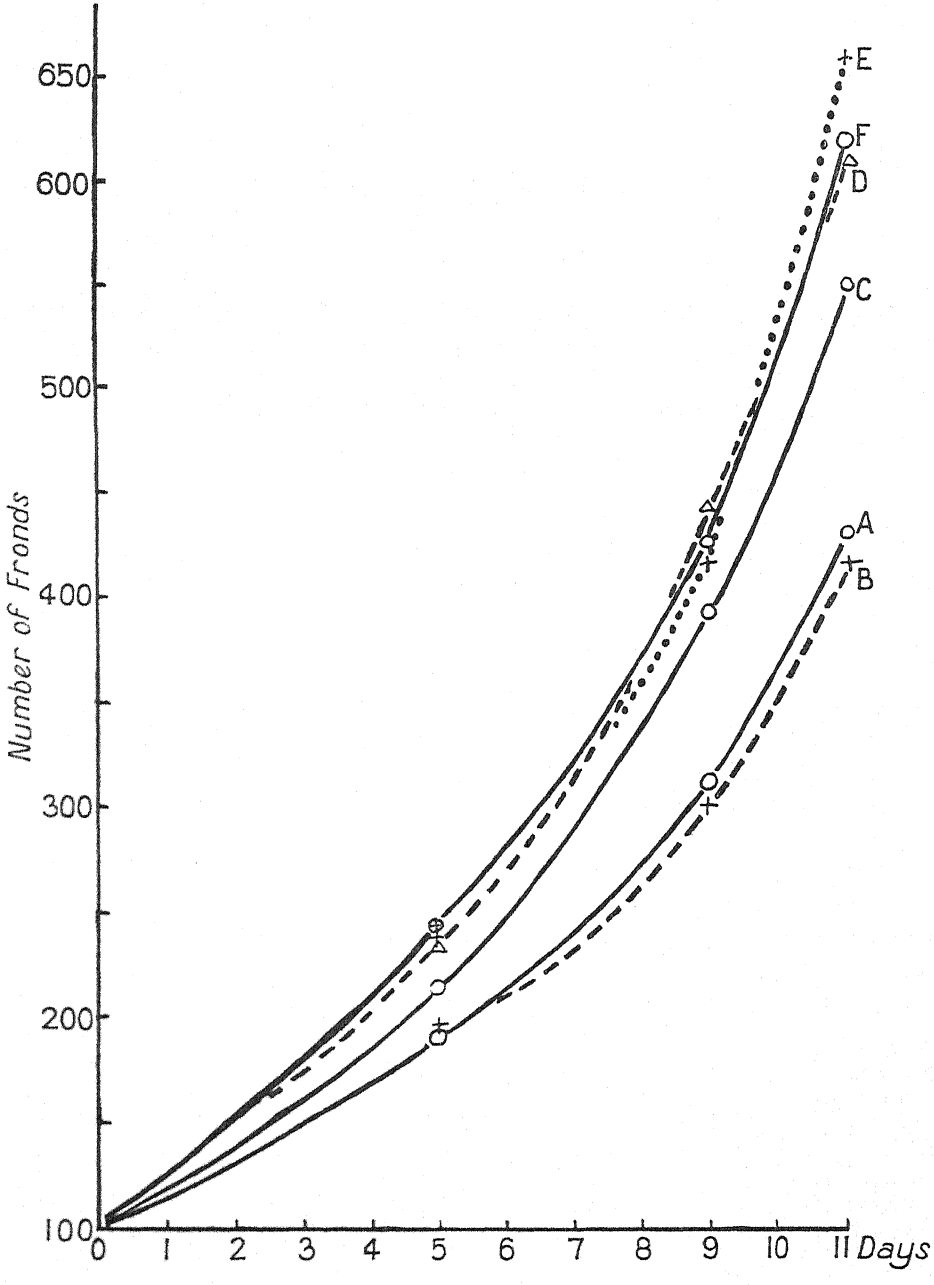


FIG. 2. Growth curves for *Lemna* in different solutions. For description see text.

of a green plant must be attributed to one of two causes, or to a combination of them:

(1) The 'formative efficiency', i. e. the amount of photosynthetic tissue formed per unit income of dry weight, is increased.

(2) The efficiency of the photosynthetic mechanism itself is increased.

With the exception of the insignificant roots, almost all the tissue in *Lemna* is photosynthetic tissue. Its 'formative efficiency', therefore, cannot change; so that the second alternative alone needs consideration. Although the frond weight has been shown by the writer to vary considerably under different intensities of light and different daily periods of illumination, the addition of organic matter does not affect its value in the least. However, the area of the fronds in solutions D, E, and F was 33 per cent. greater than the area of the control fronds.

In addition to these morphological differences, various anatomical differences between the treated and untreated fronds were found. The mean relative diameters of treated and untreated mesophyll cells were as 1·18 : 1. The relative volumes, therefore, assuming the cells to be spherical, were as 1·64 : 1. From sections, the relative thickness of treated and untreated fronds was found to be as 1·20 : 1. Assuming the fronds to be cylindrical plates, their relative volume would be: relative area × relative thickness, i. e. $\frac{1\cdot33}{1\cdot00} \times \frac{1\cdot20}{1\cdot00} = \text{approx. } \frac{1\cdot60}{1\cdot00}$. Since the volumes of treated and untreated cells are as 1·64 : 1, the ratio of the number of cells per frond is $\frac{1\cdot00}{1\cdot64} \times \frac{1\cdot60}{1\cdot00}$. To a first approximation, then, the number of cells in treated and in untreated fronds is the same.

Finally, it was observed that there were roughly 1·8 times as many chloroplasts in the cells of fronds treated with organic extract as in the cells of untreated fronds;[1] and since the number of cells in both sets of fronds is about the same, the treated fronds contain approximately 1·8 times as many chloroplasts as the untreated. This explains satisfactorily the greater photosynthetic efficiency of the treated fronds, and their consequent increased growth-rate. The effect upon growth of such an increase in the amount of chlorophyll will, of course, be cumulative, and the divergence between colonies growing with and without organic matter will become increasingly great.

It may be concluded, then, that the application of minute amounts of organic matter from horse dung increases the area of the fronds, the cell size, and the number of chloroplasts per frond. The increased amount of chlorophyll results in increased photosynthetic efficiency of the frond, and hence an increased growth-rate, which is manifested by an increase in the rate of multiplication of the fronds.

[1] These and the previous measurements are the means of twenty counts.

Previous work has shown that *Lemna* can grow indefinitely in a purely inorganic medium, so that 'auximones', in Bottomley's sense of the word, are not necessary. But it seems clearly established that minute quantities of organic matter—less than 0·02 per cent. of the mineral matter already present, and *involving an addition of nitrogen of less than 0·03 per cent. of the nitrogen already present*, can increase the growth-rate. It seems certain, too, that the action is of a catalytic nature, since any addition above a minimum dose has no further effect upon the growth-rate.

VI. Summary.

An experiment is described which was designed to discover the influence of minute quantities of organic matter upon the growth of *Lemna minor*. The strain employed had been growing in a purely inorganic medium for six months. The experiment was carried out under controlled conditions, and with precautions as to constancy of pH, avoidance of contamination, &c.

An extract of fresh horse dung was prepared, and was added in the following concentrations to a standard inorganic solution containing 1,210 parts per million of mineral matter, of which 110 parts per million were the nitrogen KNO_3:

A. Control.
B. 20 parts per mill. (by weight) of organic extract (but reduced to ash).
C. 0·2 ,, ,, ,, ,, ,,
D. 2·0 ,, ,, ,, ,, ,,
E. 20 ,, ,, ,, ,, ,,

The control solution (A) contained no organic material, but an extra three parts per million of nitrogen were added as KNO_3.

Populations of *Lemna* were grown in these solutions, and the plant number, frond number, dry weight, area, and carbon content were taken from time to time during eleven days. The growth-rate in solution C was found to be significantly greater than that in solutions A or B so the addition of minute quantities of organic matter clearly increases the growth-rate. The growth-rate in solution E did not exceed the rate in solution D; i. e. the addition of organic matter above a concentration of two parts per million results in no further increase of the growth-rate. In this respect the action of organic matter is of a catalytic nature.

The frond weight is unaffected; but the frond area and the cell size are increased by the addition of organic matter.

The number of chloroplasts per frond is increased, resulting in an increased rate of photosynthesis, and hence in an increased growth-rate.

It is concluded that organic matter is not essential for the growth of *Lemna minor*; but that minute quantities, when added to an inorganic solution, do increase the growth-rate to a striking degree.

I have to record my thanks to Professor V. H. Blackman, for his criticism and advice in the course of this work.

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Chemical Studies in the Physiology of Apples.

XI. The Relation between the Mineral Constitution of Apples and the Soil on which they are Grown.

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With nine Figures in the Text.

SOME preliminary published work (3) on the relationship between the mineral constitution of the apple and that of the soil on which it is grown showed that large differences in mineral content were related to variations in the soils, and that similar types of soils, such as silt and gravel, produced apples with similar percentages of mineral constituents. The influence of the soil on the mineral constitution of the apple has been now investigated further, and the mineral content of two varieties of apple has been compared in detail with the data of the mechanical and chemical analyses of the soils on which they were grown. The soil data are those of the West Midland Fruit Soil Survey and are available at the Ministry of Agriculture.

The apples were analysed in 1925 and 1926 and were Bramley's Seedlings and Worcester Pearmains from ten orchards in the West Midland area. The mechanical analysis, and the figures for available potash and phosphate, were given in the soil survey for all the ten soils, but a complete chemical analysis was only available for six of the soils. In the apples the total ash, potash, phosphate, lime, magnesia, and iron were estimated, using methods already described (2 and 3). Nitrogen and other estimations on the same apples have been described by Archbold (2). The work will be discussed under the following headings: (1) Seasonal Variation, (2) Influence of the Nature of Soil. (*a*) Mechanical, (*b*) Chemical.

1. Seasonal Variation.

The analyses of the apples in the two years are given in Table I, which shows that the seasonal differences are small, and not nearly so great as those caused by variations in the soil; this agrees with former analyses by

the author (2). The apples in 1925 and 1926 were not gathered from the same trees (see (2) p. 553), so that some of the differences in Table I between the analyses in the two years may not be due to season but to soil and tree differences.

It appears from Table I that the mineral content of the apples tends to be higher in 1926, which was a 'bad' season. This increase is chiefly in the percentage of phosphate and to a lesser degree in the percentage of potash. The phosphate increases on the basis of fresh weight (column *a*) are, for the apples from the orchard C 5·7 per cent., F 26 per cent., H 11 per cent., I 16 per cent., E 5·4 per cent., and B 37 per cent. The potash increases on the same basis are C 11 per cent., F 7·5 per cent., H 25 per cent., I −3·5 per cent., E −8·5 per cent., and B 23 per cent. The percentages of magnesia and lime have also increased, except for magnesia in the apples from the orchard I, and lime in those from orchard H. The disproportionately large increase in the phosphate content is shown by the fact that the percentage of phosphate in the total ash (Column *β*) has also increased considerably in all but the apples from the orchards C and H, where the differences between the two years are not significant. The increases in the percentages of phosphate in the total ash are C 1 per cent., F 19 per cent., H −1 per cent., I 28 per cent., E 17 per cent., and B 23 per cent. The percentage of potash, however, in the total ash is the same in the two years in all but the apples from the soil B. The increase in mineral content in 1926 is probably due to the effect of the larger rainfall [1] in the earlier part of that year.

The work of Greaves and Nelson (11) and Greaves and Carter (9 and 10) shows that an increased percentage of irrigation water causes an increase in the bacterial activity of the soil, and this increases the availability of the different elements in the soil and hence their absorption by the plants, and it may be assumed that the effect of increased rainfall is similar.

2. Variation due to the Soil.

Large and significant [2] differences between the percentages of mineral constituents are brought about by variations in the soil.[3] This is shown in Table I and more clearly in Table III, which gives the maximum and

[1] Total rainfall during the months of April, May, and June was:

Ross on Wye	1925	5·03″.	Bromyard	1925	5·64″.
„	1926	6·13″.	„	1926	6·75″.

During the months of April, May, June, July, and August:

Ross on Wye	1925	10·24″.	Bromyard	1925	10·52″.
„	1926	10·82″.	„	1926	11·46″.

[2] From previous determinations of the probable error, a difference greater than 10 per cent. may be taken as significant (4).

[3] The differences due to stocks were not considered, since the stocks on which the trees were grafted were not known, except that they were vigorous and not dwarfing.

TABLE I.

Analysis of Apples Grown on Different Soils.

Figures in *a* columns represent percentages referred to fresh weight.
" " *β* " " " " total ash.

Soil Bromyard Area.	Total Ash. Bramley's Seedlings 1925.	Total Ash. Bramley's Seedlings 1926.	Total Ash. Worcester Pearmains 1926.	Potash (K_2O). Bramley's Seedlings 1925. *a.*	Potash (K_2O). Bramley's Seedlings 1925. *β.*	Potash (K_2O). Bramley's Seedlings 1926. *a.*	Potash (K_2O). Bramley's Seedlings 1926. *β.*	Potash (K_2O). Worcester Pearmains 1926. *a.*	Potash (K_2O). Worcester Pearmains 1926. *β.*	Phosphate (P_2O_5). Bramley's Seedlings 1925. *a.*	Phosphate (P_2O_5). Bramley's Seedlings 1925. *β.*	Phosphate (P_2O_5). Bramley's Seedlings 1926. *a.*	Phosphate (P_2O_5). Bramley's Seedlings 1926. *β.*	Phosphate (P_2O_5). Worcester Pearmains 1926. *a.*	Phosphate (P_2O_5). Worcester Pearmains 1926. *β.*
C	0·204.	0·213.	0·238	0·1085	53·3	0·1200	55·3	0·1348	56·4	0·0226	11·10	0·0239	11·22	0·0248	10·41
D	0·222	—	0·220	0·1205	56·0	—	—	0·1204	54·7	0·0198	8·94	—	—	0·0220	10·02
A	0·235	—	0·225	0·1235	52·6	—	—	0·1241	55·1	0·0155	6.60	—	—	0·0244	10·87
F	0·192	0·200	—	0·0940	49·1	0·1010	50·5	—	—	0·0158	8·26	0·0197	9·85	—	—
G	0·197	—	0·231	0·0996	51·3	—	—	0·1268	54·8	0·0210	10·69	—	—	0·183	7·93
H arable	0·161	0·181	—	0·0866	54·0	0·1080	59·5	—	—	0·0160	9·95	0·0177	9·83	—	—
I	0·209	0·189	—	0·1150	55·0	0·1010	53·5	—	—	0·0198	9·47	0·0229	12·14	—	—
J	0·197	—	—	0·1030	53·0	—	—	—	—	0·0193	9·81	—	—	—	—
Ross Area															
E arable	0·199	0·181	—	0·1118	58·9	0·1080	59·8	—	—	0·0187	9·40	0·0197	11·04	—	—
B	0·167	0·186	0·186	0·0886	53·0	0·1083	58·3	0·1067	54·8	0·0157	9.41	0·0215	11·60	0·0162	8·84

Soil Bromyard Area.	Magnesia (MgO). Bramley's Seedlings 1925. *a.*	Magnesia (MgO). Bramley's Seedlings 1925. *β.*	Magnesia (MgO). Bramley's Seedlings 1926. *a.*	Magnesia (MgO). Bramley's Seedlings 1926. *β.*	Magnesia (MgO). Worcester Pearmains 1926. *a.*	Magnesia (MgO). Worcester Pearmains 1926. *β.*	Lime (CaO). Bramley's Seedlings. 1925. *a.*	Lime (CaO). Bramley's Seedlings. 1925. *β.*	Lime (CaO). Bramley's Seedlings. 1926. *a.*	Lime (CaO). Bramley's Seedlings. 1926. *β.*	Lime (CaO). Worcester Pearmains 1926. *a.*	Lime (CaO). Worcester Pearmains 1926. *β.*	Iron (Fe_2O_3). Bramley's Seedlings 1926. *a.*	Iron (Fe_2O_3). Bramley's Seedlings 1926. *β.*	Iron (Fe_2O_3). Worcester Pearmains 1926. *a.*	Iron (Fe_2O_3). Worcester Pearmains 1926. *β.*
C	0·0051	2·49	0·0058	2·74	0·0082	3·44	0·0032	1·69	0·0047	2·20	0·0041	1·73	0·00030	0·140	0·00038	0·160
D	0·0055	2·49	—	—	0·0074	3·37	0·0037	1·68	—	—	0·0054	2·24	—	—	—	—
A	0·0053	2·49	—	—	0·0082	3·65	0·0033	1·39	—	—	0·0047	2·09	—	—	—	—
F	0·0054	2·83	0·0060	3·00	—	—	0·0036	1·88	0·0041	2·05	—	—	0·00037	0·185	—	—
G	0·0056	2·83	—	—	0·0073	3·17	0·0037	1·86	—	—	0·0055	2·38	—	—	0·00034	0·147
H arable	0·0057	3·52	0·0060	3·31	—	—	0·0037	2·32	0·0027	2·03	—	—	—	—	—	—
I	0·0055	2·64	0·0037	1·97	—	—	0·0027	1·27	0·0033	1·77	—	—	0·00045	0·237	—	—
J	0·0050	2·57	—	—	—	—	0·0023	1·19	—	—	—	—	—	—	—	—
Ross Area																
E arable	0·0048	2·40	0·0052	2·88	—	—	0·0031	2·00	0·0037	2·06	—	—	0·00030	0·166	—	—
B	0·0043	2·56	0·0056	2·88	0·0062	3·38	0·0035	2·10	0·0039	2·10	0·0070	3·82	0·00049	0·263	0·00019	0·105

minimum percentages of each of the mineral constituents found in the same variety and season, grown on different soils. The difference between the two, calculated as a percentage on the minimum value, is also given; this is from 35 to 50 per cent. in Bramley's Seedlings in 1925. It is therefore extremely important in any comparison of the mineral constituents of fruit that the soil factor should be considered, since in apples it may cause differences of the order of 50 per cent. in the analyses of the individual element.

TABLE II.

Analysis of the Soils.

Figures in Columns I represent 'available' (soluble in 1 % citric acid) potash or phosphate.

Figures in Columns II represent 'relative solubility' (available/soluble in 20 % HCl) potash or phosphate.

Soil.	Potash (K_2O).		Phosphate (P_2O_5).		Magnesia (MgO).		Lime (CaO).	
Bromyard Area.	I.	II.	I.	II.	Sol. in 20% HCl	Total.	Sol. in 20 % HCl	Total.
C	0·0105	0·0108	0·0212	0·354	0·54	2·78	0·82	1·57
D	0·0092	0·0122	0·0027	0·032	0·42	0·67	0·85	1·41
A	0·0083	—	0·0161	—	—	—	—	—
F	0·0038	0·0044	0·0060	0·100	1·73	2·50	0·76	1·08
G	0·0078	0·0084	0·0141	0·176	1·80	2·61	0·30	1·23
H arable	0·0122	—	0·0426	—	—	—	—	—
I	0·1020	—	0·0236	—	—	—	—	—
J	—	—	0·0469	—	—	—	—	—
Ross Area								
E arable	0·0134	0·0272	0·0350	0·350	0·64	1·45	0·48	0·95
B	0·0062	0·0182	0·0152	0·277	0·63	1·21	0·57	1·06

The figures given are the means of the figures for soil and subsoil (1–18 in.).

(a) *The Effects of the Mechanical Structure of the Soil on the Mineral Constitution of the Apple.*

The ten soils analysed fall into two groups, the two orchards (B and E) in the Ross area and the eight orchards (A, C, D, F, G, H, I, J) in the Bromyard area. The Ross soils are more sandy, consisting in over 60 per cent. coarse and fine sand, and only 6–8 per cent. clay. The Bromyard soils have from 12 to 18 per cent. of clay and only about 25 per cent. of coarse and fine sand. In Tables I and II the soils are arranged in the order of decreasing content of clay and fine silt, using the mean of the figures for soil and subsoil. The soils C, D, and H are very similar in mechanical composition, as are also the soils G, H, I, and J. Soil D has much the highest 'moisture' content, that of 3·5 per cent. (mean of analyses taken at depth of 1–30 in. from the chemical analysis), while the soil F, with the next highest

'moisture', has only 1·9 per cent. Apples grown on the Bromyard soils, containing more clay and having a higher 'moisture' content (see Table IV) tend to give a larger percentage of total ash than the Ross apples, thus for Bromyard apples the mean value for total ash is 0·200 per cent., and 'moisture' is 2·1 per cent., while for Ross apples the mean value for total ash is 0·181 per cent., and 'moisture' 1·2 per cent.

(b) *The Effect of the Chemical Composition of the Soil on the Mineral Constitution of the Apples.*

In order to compare the composition of the apple with that of the soil, the mean soil survey figures of the analyses for the soil and subsoil (1–18 in. and for moisture content 1–30 in.) are taken (see Table II), for according to Lemmerman, Weismann, and Eckl (15) the food supply in the subsoil cannot be ignored in plant nutrition studies, and this would obviously be even more important in the case of trees. In Figs. 1–9 the percentages of the different constituents of the ash in the apple have been plotted against their percentages in the soils. In the analyses of potash and phosphate in the soils the 'available' (soluble in 1 per cent. citric acid), the percentages soluble in 20 per cent. hydrochloric acid, and also total percentages are given. It was thought that an examination of these figures in order to discover the best basis of comparison with the mineral composition of the apple would be useful in plant nutrition and manuring problems. The figures for 'available' potash and phosphate do not of course represent the true availability of these elements. The work of Lemmermann and Fresenius (13) and Lemmermann, Fresenius, and Lesch (14) on the phosphoric acid requirement of soils by the 'relative solubility' of the phosphate (the ratio of phosphate soluble in 1 per cent. citric acid to total phosphate)[1] showed that it was a better criterion of availability than the so-called 'available' phosphate. Engels (5 and 6) states that Lemmermann's 'relative solubility' method gives results that are in accord with the response of soils to phosphatic manuring, and also agrees well with determinations of the phosphate requirements of soils by Neubauer's seedling method.

The present series of results as shown in the graphs gives some support to these conclusions. In Fig. 1, where the percentage of phosphate in the Bramley's Seedling apples in 1925 is compared with 'available' phosphate in the soil, the points are very scattered, showing little relationship. However, in Fig. 2, where the percentage in the apple is compared with the 'relative solubility' of phosphate, there are indications of relationship. The

[1] Some workers (see Van der Spuij (17) use the (1) ratio of phosphate soluble in 0·5 per cent. citric acid to that soluble in hot 10 per cent. hydrochloric acid, or (2) that soluble in 1 per cent. citric acid to that soluble in 20 per cent. hydrochloric acid. In this paper (2) is used.

TABLE III.

Maximum and Minimum Percentages of Mineral Constituents in the Fresh Weight found in Analyses of Bramley's Seedling Apples (1925 and 1926) and of Worcester Pearmain Apples (1926) grown on different Soils.

Season and Variety.	Total Ash.		Potash.		Phosphate.		Magnesia.		Lime.	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Bramley's Seedling, 1925 .										
10 samples . . .	0·16	0·23	0·087	0·123	0·016	0·023	0·0043	0·0057	0·0024	0·0037
Difference	43 %		41 %		43 %		33 %		54 %	
Bramley's Seedling, 1926 .										
6 samples . . .	0·17	0·20	0·101	0·120	0·018	0·024	0·0037	0·0060	0·0033	0·0047
Difference	18 %		19 %		33 %		62 %		42 %	
Worcester Pearmains, 1926										
5 samples . . .	0·18	0·23	0·107	0·135	0·016	0·024	0·0062	0·0082	0·0041	0·0070
Difference	28 %		26 %		50 %		32 %		71 %	

Differences of more than 10 % may be taken as significant.
The differences per cent. are calculated on the lower values.

TABLE IV.

Showing the Variation of the Relative Solubility of Phosphate in the Soil with the Silica Content and Inversely with the Moisture, Clay, Organic Matter, Iron and Aluminium Content.

Soil.	Relative Solubility of Phosphate.	'Moisture' mech. anal.	'Moisture' chem. anal.	Clay Content.	Organic Matter (loss. on ignit.) mech. anal.	Organic Matter (loss. on ignit.) chem. anal.	SiO_2.	Fe_2O_2. Total.	Fe_2O_2. Sol. 20 % HCl.	Al_2O_3. Total.	Al_2O_3. Sol. 20 % HCl.
E	3·89	1·3	1·1	7·5	1·9	3·4	81·8	2·4	2·6	7·8	4·0
B	1·60	2·1	1·3	19·4	3·3	2·8	78·5	2·3	2·1	4·8	2·8
C	0·91	2·7	1·4	18·0	4·3	4·5	66·2	4·0	4·1	15·6	8·8
G	0·70	3·9	1·8	11·5	4·5	4·9	68·8	6·8	6·1	8·2	5·3
F	0·63	3·0	1·9	17·0	4·0	4·6	67·6	5·5	4·5	12·4	7·7
D	0·16	6·0	3·5	17·0	5·0	5·0	67·2	4·1	4·1	11·9	5·0

The figures given are the means of the figures for soil and subsoil (1 – 18 in.) and for moisture content (1 – 30 in.).

results tend to fall into two series, according to the two types of soil. The Bramley's Seedlings in 1925 grown on the sandy Ross soils (B and E) contain less phosphate compared with the 'relative solubility' in the soil than those grown in the Bromyard area, but for the same type of soil the phosphate content varies with the 'relative solubility'. The comparison in 1926 is incomplete, since owing to the failure of the crop, the apples were only obtained from four out of the six orchards whose soils had been completely analysed; further the availability of phosphate in the soil would probably be slightly different in 1926. However, the 1926 figures give some support to the results of the previous year. In both the Bramley's Seedlings and Worcester Pearmains of 1926 the value of the ratio of phosphate in the apple to the 'relative solubility' in the soil is higher in the Bromyard area, being 0·101 for the three samples of Bromyard Worcester Pearmains, and only 0·058 for the Ross apples, and for two samples of Bromyard Bramley's Seedlings, 0·130, compared with a value of 0·063 for the Ross area apples. The difference between the phosphate content of the apples from the two areas may be partly due to the phosphate reserves ('total' phosphate) which are less in the Ross soils, the mean value being 0·094 per cent., while for the four soils in the Bromyard area the value is 0·17 per cent. However, the chief causes of difference between the apples grown in the two areas are most probably the differences in the mechanical composition and water content of the soil (see p. 820 and Table IV). It will be noticed that the apples from the soil D, which has the highest 'moisture' content, always contain a high percentage of phosphate compared with the phosphate content of the soil (see Figs. 1 and 2). It is also of interest that in both seasons the rainfall (see note [1] on p. 818) in the Ross area is slightly lower than in the Bromyard area.

The importance of the 'relative solubility' of phosphate in the soil on the composition of the fruit has been shown, so it may be interesting to consider briefly the factors that may affect the availability of phosphate to the plant. Lemmermann, Weismann, and Semmet (16) state that silica in the soil indirectly increases the amount of phosphate which can be taken up, by exerting a solvent action on the phosphate present, and rendering it more easily available to the plant. The work of Gile and Smith (7) also supports this conclusion. Ingram (12) found that the availability of phosphate was closely related inversely to the iron, aluminium, and organic content of the soil, and also points out that low availability may be due to the absorption powers of organic and inorganic colloids, especially those of iron and aluminium. In Table IV the values for the 'relative solubility' of phosphate may be compared with the moisture, clay, organic matter, silica, iron, and aluminium contents for the six soils which were completely analysed, and it will be seen that the figures give striking evidence in support of the view that a high silica content, together with low values for

iron, aluminium, and organic matter, increase the 'relative solubility' of the phosphate, and so probably the availability to the plant.

In Table IV the soils are arranged in order of decreasing values of

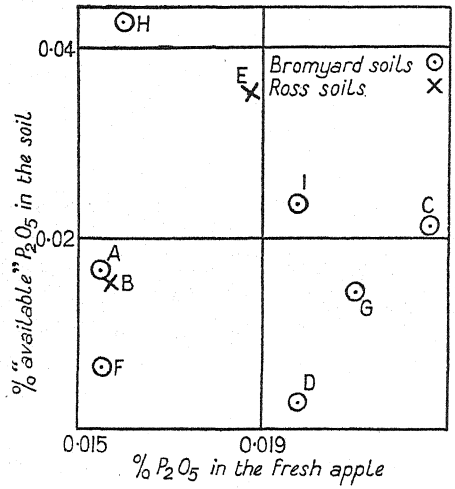


FIG. 1.

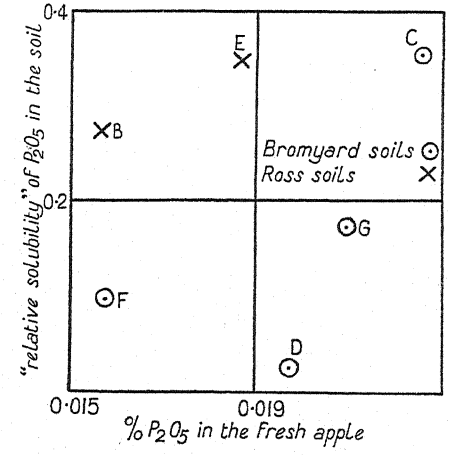


FIG. 2.

FIGS. 1–2. 1. Effect of the 'availability' of phosphate in the soil on the percentage of phosphate in the fresh apple. Bramley's Seedling, 1925. 2. Effect of the 'relative solubility' of phosphate in the soil on the percentage of phosphate in the fresh apple. Bramley's Seedlings, 1925.

'relative solubility' of phosphate, and with very few exceptions this is also the order of increasing percentages of clay, organic matter, and iron and aluminium oxides, and of decreasing silica content. It will be seen that the soil E, with the highest value for the 'relative solubility' of phosphate, has the highest silica content, and the lowest content of clay, organic matter, and

of moisture: while the soil D with the lowest 'relative solubility' of phosphate, has a low silica content and the highest percentage of organic matter and moisture.

Potash. Using the same basis of comparison as with phosphate the relations between the potash in the soil and in the apple are found to be

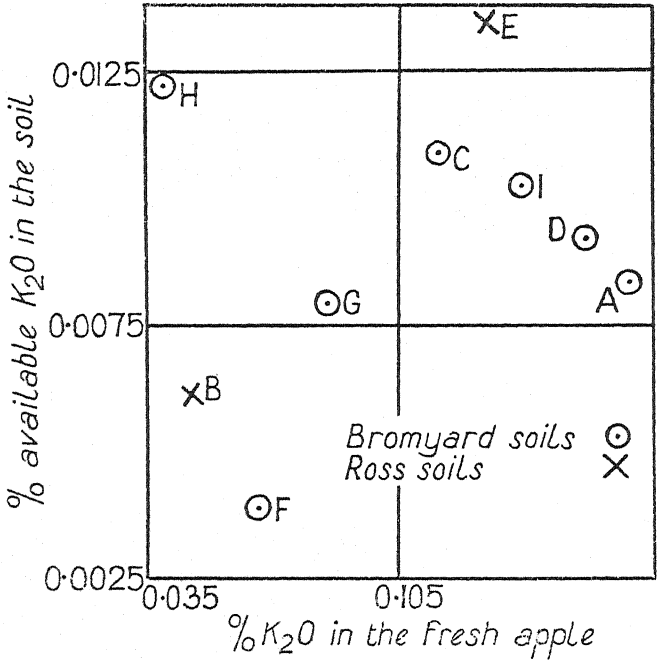


FIG. 3. Effect of 'availability' of potash in the soil on the percentage in the fresh apple. Bramley's Seedlings, 1925.

similar and much closer. It is shown in Fig. 3 that there is very little relation between the percentage of 'available' potash in the soil and the 'fresh weight' percentages in the apple. However, in Fig. 4, where the 'relative solubility' of potash is compared with these percentages, the group arrangement into series corresponding to the two types of soils is much more definite and striking than with phosphate. It is also to be noted that the ratio between the percentages of potash in the apple and the soil is lower in the apples from the Ross area; this cannot be due to any difference in potash reserves, which are similar in all the soils, but must be related to the difference in mechanical composition of the soils of the two areas. The relation between the potash content of the soil and the apples is not so definite in 1926 for the reasons already stated.

Although the available potash shows no clear correlation with the fresh weight percentages in the apple, yet Fig. 5 indicates that it influences the

percentage of potash in the ash of the apple, high values of potash in the ash being associated with high values for available potash in the soil.

Magnesia. The relation between the magnesia content of the soil and that of the apple is shown graphically in Figs. 6 to 8. In the soil

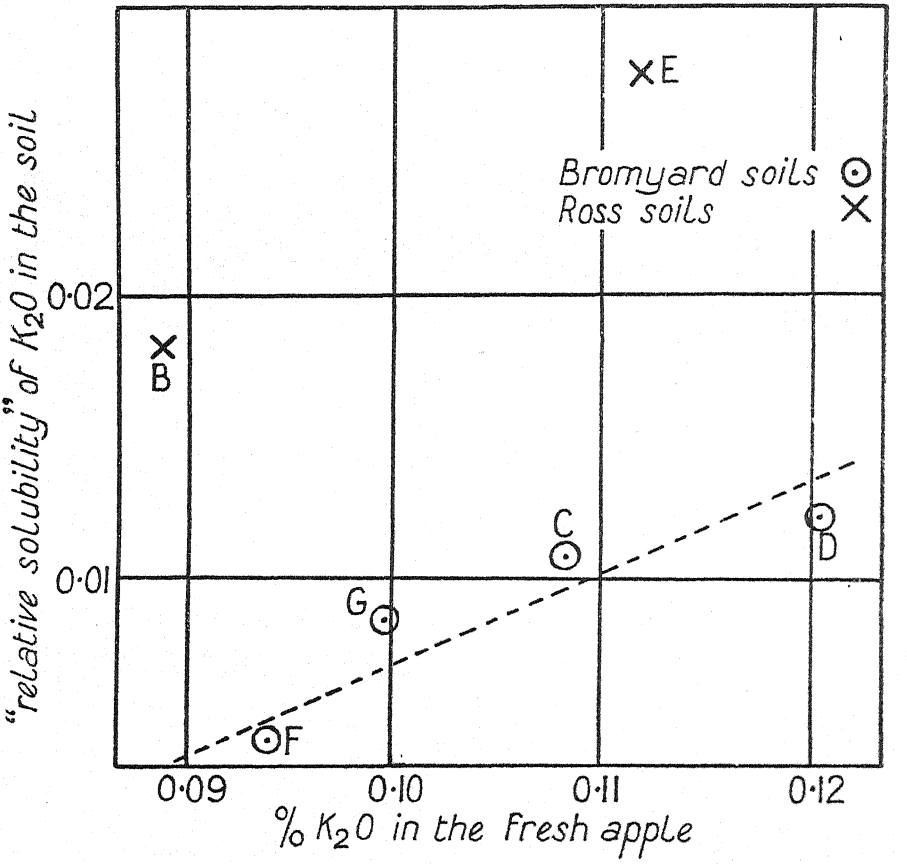


FIG. 4. Effect of the 'relative solubility' of potash in the soil on the percentage in the fresh apple. Bramley's Seedlings, 1925.

analyses the figures for total magnesia and that soluble in 20 per cent. hydrochloric acid are given. Fig. 6 shows that there is a tendency for the percentage of magnesia in the fresh apple to be governed by the percentage of total magnesia in the soil. The apples both in 1925 and 1926 from the soil D which had very high moisture content (see Table IV) contain a larger percentage of magnesia than would have been expected from the magnesia content of the soil, as was found also of the phosphate content (see Figs. 1 and 2). Figs. 1 and 8 (Bramley's Seedlings 1925 and 1926) show that, as with potash, the percentage of magnesia in the ash of the apple is related to the percentage in the soil.

Lime. The percentage of lime in the apple is also regulated by the supply in the soil, as is shown in Fig. 9. In 1925 the apples from the soil C were exceptional in having a low percentage of lime while there was a high percentage in the soil, but in 1926 this did not occur. It is interesting to

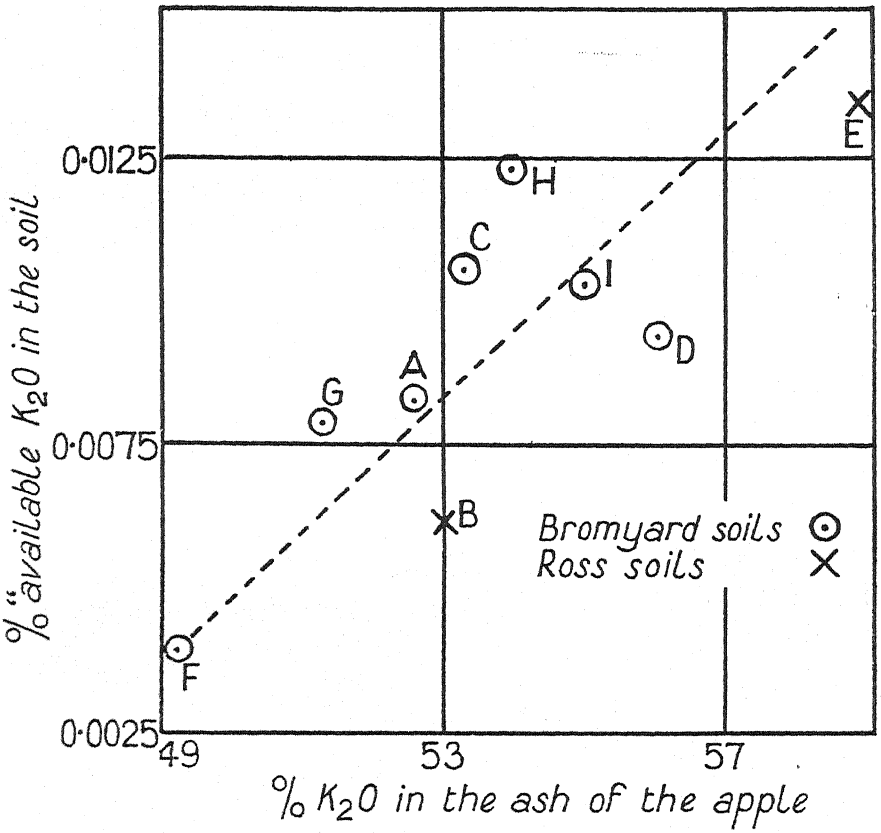


FIG. 5. Effect of the 'availability' of potash in the soil on the percentage in the ash of the apple. Bramley's Seedlings, 1926.

note that Ginsburg and Shive (8) found a definite correlation between the calcium content of beans and that of the culture solutions in which they were grown.

Iron. The amount of iron taken up is so small that there must always be a considerable excess in the soil. From the results of 1926, when in four cases only could the analysis of the apples be compared with the iron content of the soil, it seems that the percentage of iron in the apple is not governed by the total amount in the soil. It is possible, however, that the percentage of iron in the soil solution may be related to the percentage in the apple.

Ratio of Mineral Constituents. The importance of the proportion

between the different mineral constituents of the nutrient medium has been shown by many workers. Wallace (18) has shown the importance of the potash-nitrogen ratio in the soil on the intake of both potash and nitrogen.

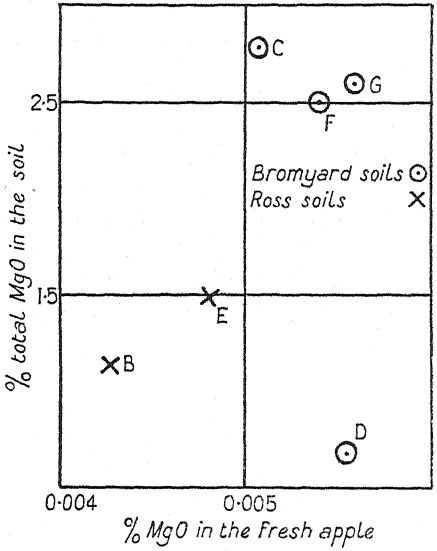


FIG. 6. Effect of the 'total' magnesia in the soil on the percentage in the fresh apple. Bramley's Seedlings, 1925.

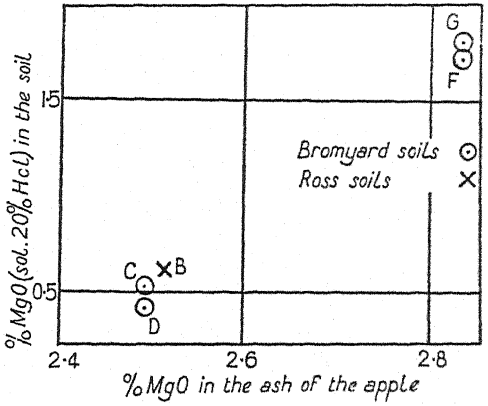


FIG. 7. Effect of the percentage of magnesia (sol. in 20 per cent. HCl) in the soil on the percentage in the ash of the apple. Bramley's Seedlings, 1925.

The analyses show a relation between the potash-nitrogen ratio in the soil and in the apple, but no importance can be attached to this, since both the potash and nitrogen are individually related to the percentage in the soil (see Figs. 4 and 5 and Archbold (2) p. 552). The absorption of both potash and phosphate can also be influenced by the amount of lime in the soil on account of its effect on soil reaction.

Relation between the Mineral Composition of the Apple and its Keeping Qualities. The results of storage tests with Bramley's Seedling apples carried out by the Food Investigation Board for the Fruit Soils Survey Committee of the Ministry of Agriculture and Fisheries (1) indicate that

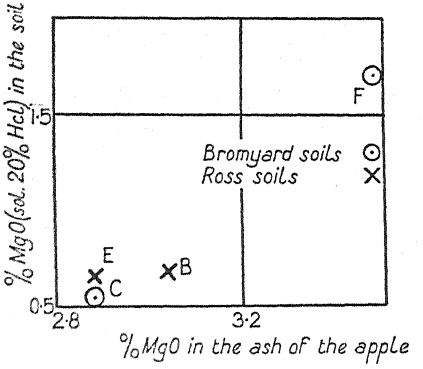


FIG. 8. Effect of the percentage of magnesia (sol. in 20 per cent. HCl) in the soil on the percentage in the ash of the apple. Bramley's Seedlings, 1926.

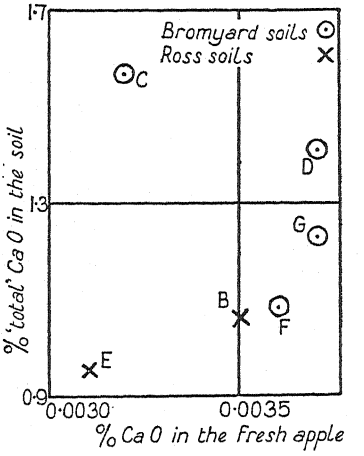


FIG. 9. Effect of the percentage of total lime in the soil on the percentage in the fresh apple. Bramley's Seedlings, 1925.

a correlation exists between keeping quality and 'available' potash and 'available' phosphate in the soil. Thus a very interesting relation is apparent, for the percentage of potash in the ash of the apple, and to a certain extent the 'fresh weight' percentage in the apple, is regulated by the percentage of 'available' potash in the soil; therefore good keeping qualities are associated with high percentages of potash in the apple (see (3) and (4)). Keeping qualities of the apple are also affected by the phosphate content, for the apples from the two soils B and F which kept the worst contained the lowest percentages of phosphate.

Other work undertaken at the Imperial College for the Food Investigation Board (2) has shown that low nitrogen and high sugar content are also related with good keeping qualities; this suggests that nitrogen and potash are inversely related, but no clear indications of correlation were shown in these apples since the data were insufficient for the partial correlations to be worked out.

SUMMARY.

1. The relationship between the mineral composition of the apple and that of the soil was investigated for ten different soils.

2. The percentages of potash in the apple show a definite relationship

with the amount in the soil, and there are indications of similar relationship between the percentages of phosphate, magnesia, and lime. The 'relative solubility' and 'availability' of phosphate and potash in the soil are compared with the fresh weight percentages in the apples (Figs. 1–4) and the 'relative solubility' shown to be a better basis of comparison, being directly related to the percentages in the fresh apple for similar types of soil.

3. In cases where the chemical composition is similar the uptake of mineral constituents is affected by differences in the mechanical composition of the soil.

4. Good keeping qualities are associated with high percentages of 'available' potash and phosphate in the soil, and with high percentages of potash and phosphate in the apple.

The writer wishes to thank Professor V. H. Blackman, under whose direction the work has been carried out, and Dr. D. Haynes and Dr. H. Harwood, for their kind advice and criticism.

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The Ovule and Gametophytes of Athrotaxis selaginoides, Don.

BY

W. T. SAXTON

AND

J. DOYLE.

With twenty Figures in the Text.

COMPARATIVELY few genera of Conifers now remain of which nothing is known regarding the gametophytes and embryo. One of these is the Tasmanian genus *Athrotaxis*. Good specimens of three of the four species are to be seen at Kilmacurragh Estate, Co. Wicklow, and during a visit there in July, 1928, the writers were struck by the fact that two of the three were forming cones fairly freely. Subsequently one of us, with the kind permission of the resident, made collections of *A. selaginoides* at intervals of about ten days to a fortnight from February to July 1929. At times this interval proved rather too long to obtain a close series, with the result that there are some gaps in the stages obtained which it will be impossible to fill until next year. Some of the results obtained are, however, so unexpected and so unique, that it seems desirable to place them on record. We hope to be in a position to present further details at a later date.

The young ovule is small and the micropyle very short. A row of archesporial cells may be seen, probably derived from a smaller number or possibly one, though in the latter case we have no evidence that this cell was hypodermal. Fig. 1 indicates the structure of the ovule in longitudinal section at the earliest stage seen. The lowest cell of the archesporial row becomes the megaspore mother-cell (Fig. 2). This divides to form the usual linear tetrad, while a few surrounding cells break down and absorb stains very deeply (Figs. 3 and 4). The lowest cell of the tetrad is functional, and early stages of the prothallus may be seen about half-way down the nucellus (Fig. 5). The ovule is by this time definitely winged, though still very small; pollination has taken place, and the micropyle has been closed in the usual way by specialized micropyle-closing cells (Figs. 6 and 7).

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The behaviour of the pollen is the first indication that there are some unusual features in development. The tube begins to form either laterally

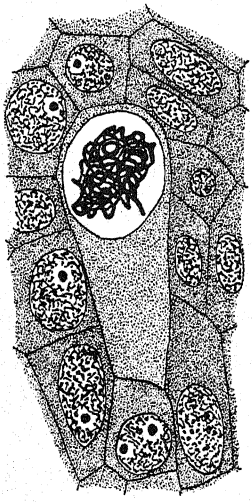


FIG. 2.

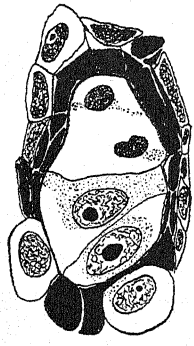


FIG. 4.

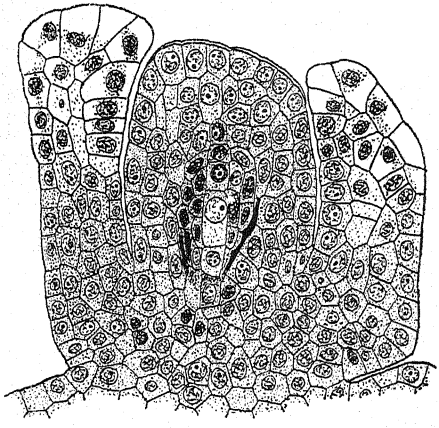


FIG. 1.

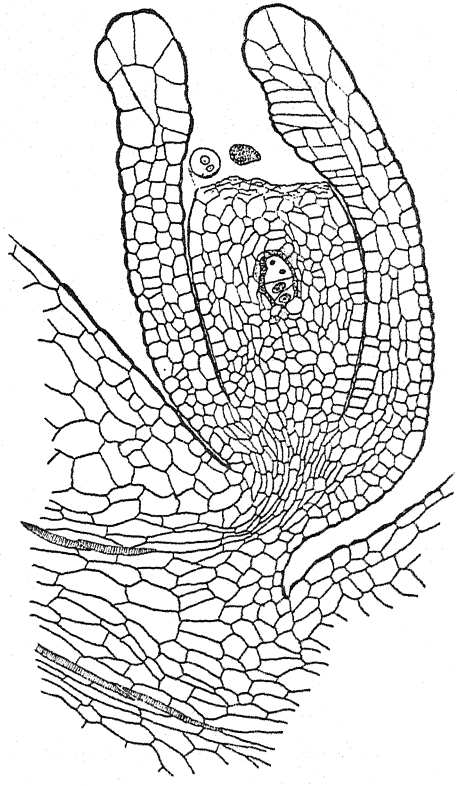


FIG. 3.

FIGS. 1–4. 1. Median longitudinal section of a very young ovule showing row of archesporial cells. The lowest becomes the megaspore mother-cell. Feb. 19, 1929. × 300. 2. Megaspore mother-cell recovering from synizesis. March 17. × 1,000. 3. Longitudinal section of ovule showing linear megaspore tetrad. April 11. × 150. 4. Part of preceding. × 530.

or upwards, but never downwards into the nucellus. At a later date the prothallial cavity occupies most of the upper half of the nucellus, only a very small part of the latter remaining above it. The pollen-tube rapidly grows across the top of the nucellus, and then down between the integument and

nucellus. At some point, commonly about half-way down the length of the young prothallus, it breaks through the nucellar tissue and continues its downward growth in contact with the megaspore membrane. Its tip grows to a point level with, or slightly below, the base of the prothallus (Fig. 8).

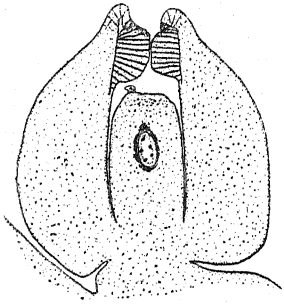


FIG. 5.

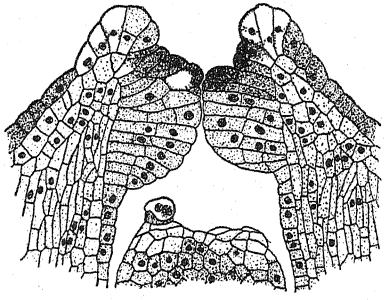


FIG. 6.

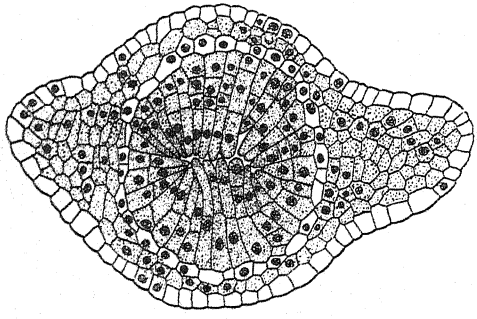


FIG. 7.

FIGS. 5–8. 5. Median longitudinal section of ovule in the plane of the wings, shortly after pollination, showing germinating pollen-grain, micropyle closing cells, and 8-nucleate prothallus. April 25. × 55. 6. Part of preceding. × 140. 7. Transverse section of a similar ovule, in the plane of the micropyle-closing cells. April 25. × 150. 8. Longitudinal section of older ovule, showing position of pollen-tube. May 21. × 90.

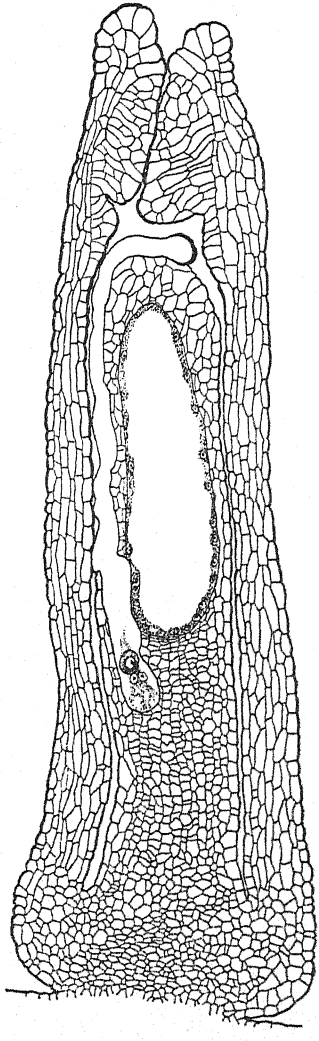


FIG. 8.

Commonly either one (Fig. 8) or two (Figs. 9 and 10) pollen-tubes may be found in an ovule, and anything up to five normal pollen-tubes have been seen occasionally in later stages. Unpollinated ovules are similar in other respects, though we have evidence that the osmotic condition inside the prothallial cavity is different when no pollen-tube is present. The structures usual in Conifers are seen in the tip of the pollen-tube, namely the body-

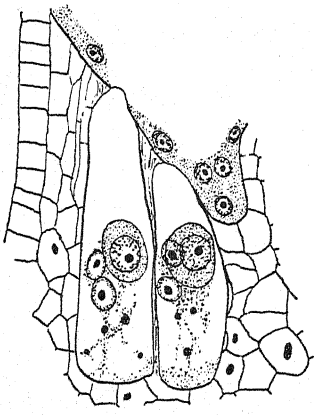


FIG. 10.

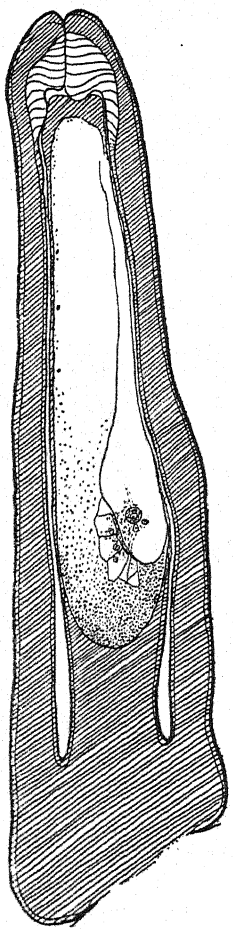


FIG. 11.

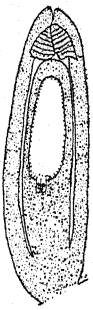


FIG. 9.

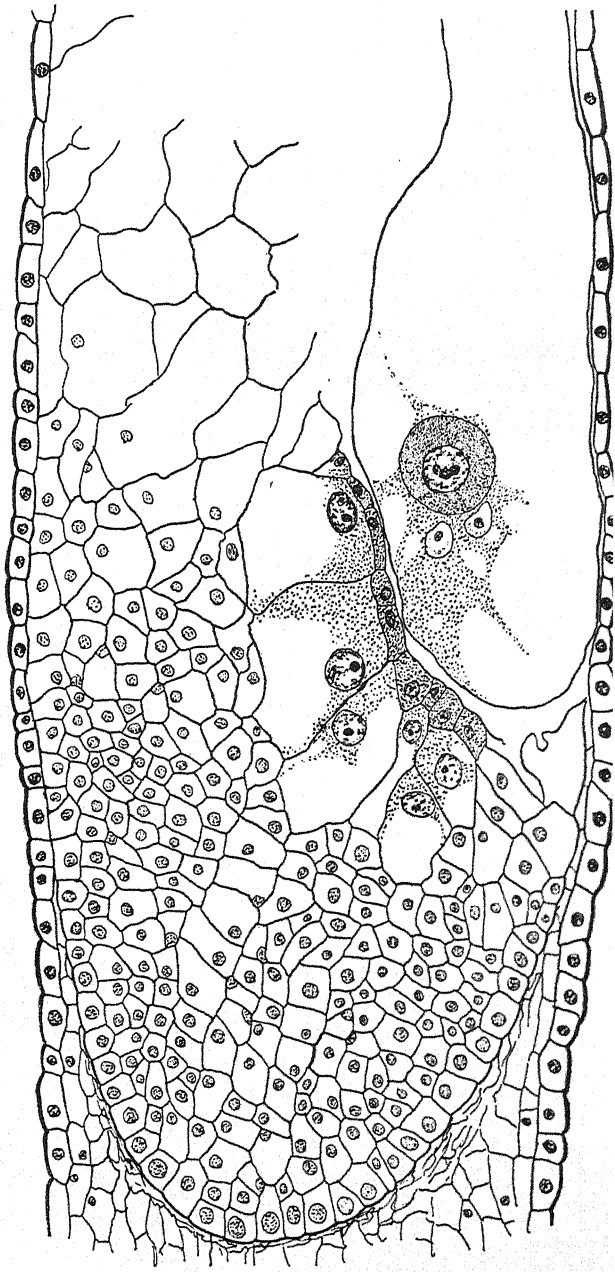


FIG. 12.

FIGS. 9–12. 9. Diagram of longitudinal section of an ovule with two pollen-tubes. May 21. × 24. 10. Part of preceding. × 285. 11. Longitudinal section of older ovule, showing pollen-tube and archegonia. June 4. × 40. 12. Part of preceding. × 260.

cell and the two sterile nuclei. The body-cell subsequently divides to form two equal, oval male cells (Fig. 15). Between May 21 and June 4 various changes occur in the pollinated ovules, of which cell-formation is the most important. At the same time lateral and downward extension of the pro-

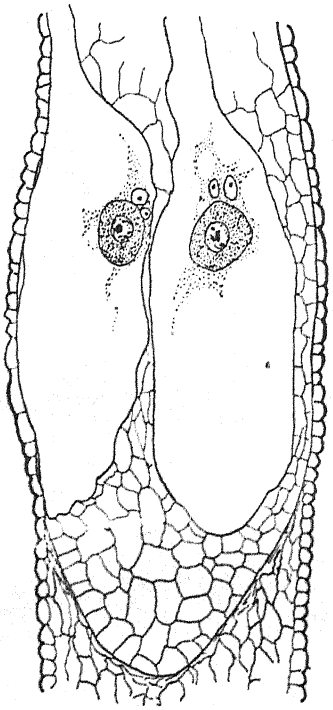


FIG. 13.

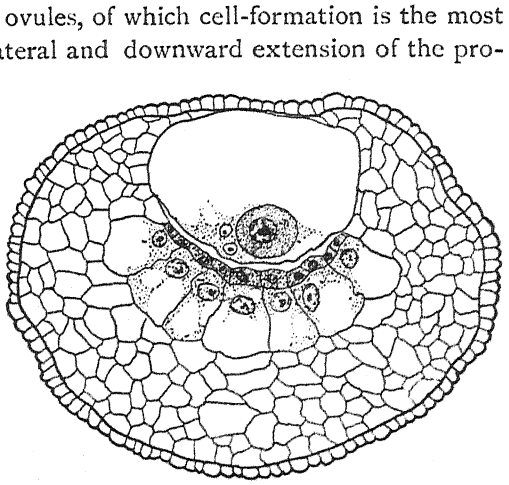


FIG. 14.

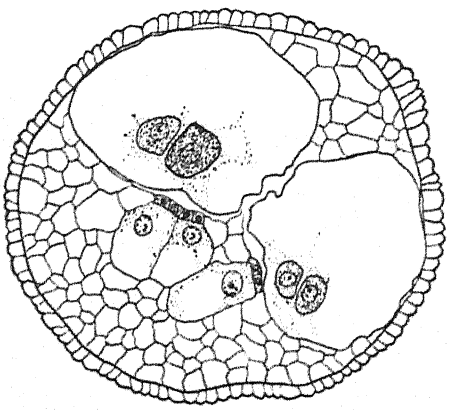


FIG. 15.

FIGS. 13-15. 13. Longitudinal section of similar ovule showing two out of the four pollen-tubes present. June 4. × 150. 14. Transverse section of similar ovule with one pollen-tube and archegonial complex. June 4. × 150. 15. Transverse section of similar ovule with two pollen-tubes and one archegonial complex. The body-cells have divided to form two male cells each. June 4. × 150.

thallus into the nucellus takes place, and the pollen-tubes pass through the megaspore membrane into the prothallial cavity. Except at the extreme base and apex, the prothallus completely absorbs the tissue of the nucellus up to, but not including, the external layer of cells. Wall-formation only takes place towards the base of the prothallus, the upper half or three-quarters remaining as one large vacuole around which only the original lining layer of cytoplasm and nuclei remains. The tips of the pollen-tubes

swell up considerably and are embedded in the basal tissue of the prothallus, which apparently grows up round them (Figs. 11–13). Archegonia varying in number from a very few up to about twenty-five may be formed in a single complex; they are small, and have a neck of two cells, always present and easily seen (Figs. 12, 14, 15, 16). The archegonia invariably

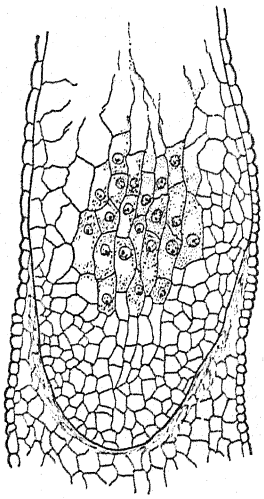


FIG. 16.

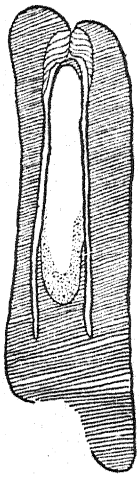


FIG. 17.

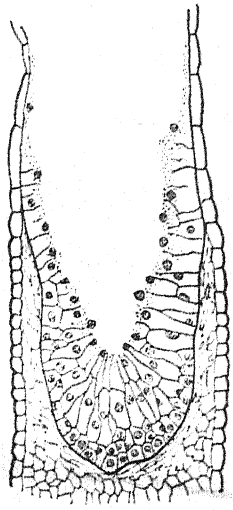


FIG. 18.

FIGS. 16–18. 16. Tangential section through an archegonial complex. June 4. × 100. 17. Longitudinal section of an ovule without any pollen-tube, showing very regular basal development of tissue. June 4. × 25. 18. Part of preceding. × 100.

abut on the pollen-tubes and may usually be seen, especially in transverse section, to be formed from deep-seated cells of the prothallus as in *Widdringtonia* (4), *Callitris* (5), and *Actinostrobus* (6). This is not always clear in longitudinal section, and where a pollen-tube lies partly external to the prothallus the archegonia may be external also, but this position seems the exception rather than the rule. No ventral canal nucleus has been seen, but the series obtained was by no means close enough to infer its absence. There is very little trace of jacket-cells round the archegonial group.

No other case is known, among Conifers, where cell-formation is confined to the basal end of the prothallus. In *Sequoia sempervirens* (1) cell-formation begins sooner, both at the upper and lower ends than in the middle of the sac, but ultimately the whole becomes cellular. In *Widdringtonia* (4) the arrangement of archegonia is sometimes comparable, but in this case cellular tissue invariably extends up to the pointed apex.

In prothalli which contain pollen-tubes, the method of formation of prothallial tissue is usually much obscured on account of the large size of

the swollen tips of the tubes. Where four are present they almost fill the base of the prothallus, as a rule, and the tissue is confined to the small interstices between them (Fig. 13). In such cases archegonia are apparently absent, perhaps because there is not room for them to develop. On the other hand, where no pollen-tube is present, the method of cell-formation

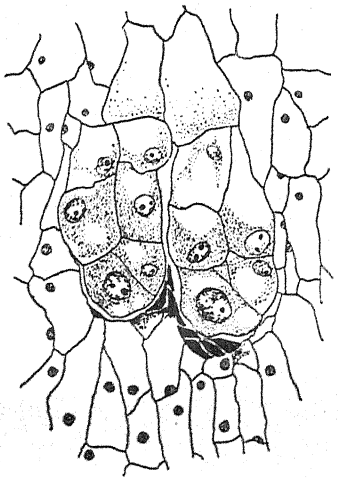


FIG. 19.

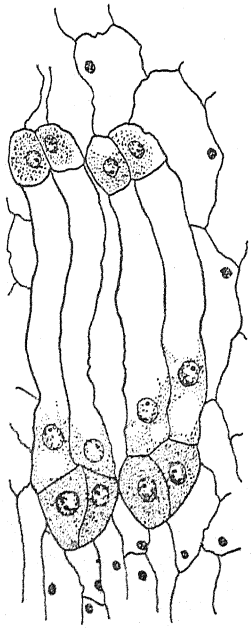


FIG 20.

FIGS. 19, 20. 19. Part of a longitudinal section of an ovule after fertilization, showing two proembryos. June 17. × 215. 20. Part of a similar section, showing two four-celled embryos, each borne on a four-celled suspensor. June 17. × 215.

by ingrowth of primary cells is exceedingly regular. These primary cells form a small basin-shaped mass of tissue, occupying only about a quarter of the total length of the prothallus (Figs. 17 and 18).

At a later date a compact cylinder of prothallial tissue continues to penetrate the base of the nucellus, and terminates upwards in the remains of the large apical vacuole. This growth leaves the fertilized archegonia further from the base of the prothallus, and the embryos then grow down into the basal cylinder, so that ultimately the structure of the seed becomes very similar to that of other Conifers. Details of fertilization and early proembryo formation are lacking at present, but a few proembryos have been seen (Fig. 19). These completely fill the archegonial cavity, and appear very similar to those of *Sequoia sempervirens* (3). The young embryos which develop from them are shown in Fig. 20.

While the actual details of development, as has been seen, differ considerably from those of both species of *Sequoia*, the results obtained tend

to support the view, based on resemblances in the structure of the cones of the two genera, that they are fairly closely related. It is not yet certain, however, whether the peculiarities described in this paper are of special systematic value, as only *A. selaginoides* was collected this season. There were no cones present on the trees of *A. cupressoides*, and, though fairly numerous on *A. laxifolia*, they were so high up as to be inaccessible. We hope it may be possible during 1930 to obtain sufficient stages from one or other (or both) of these species to indicate whether the developmental details are generic or specific in their application, as well as to fill in details hitherto lacking in regard to the species now under consideration.

We desire to record our hearty thanks to H. Digges La Touche, Esq., for permission to collect the material, to Mr. Fleming, Head Gardener at Kilmacurragh, for yielding us every facility, and to Miss L. E. Hawker for drawing Figs. 8 and 11–20.

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NOTE.

TWO SIMPLE METHODS FOR MEASURING RESPIRATION RATES.

Method I.

The apparatus and method for measuring gaseous exchanges during respiration now to be described were devised originally to meet the requirements of a large advanced class in plant physiology. The advantages claimed are as follows: (1) Only simple standard chemical apparatus is required. (2) In contrast with most other quantitative methods very little bench-room is occupied—an important consideration in a crowded class. (3) The setting up of the apparatus and the taking of the preliminary readings should occupy less than an hour, after which the apparatus can be left without attention from one to several days until it is convenient to take the final readings, which should occupy about half an hour. (4) The different types of respiration found in plants, such as that of seedlings when the reserve material consists of starch or of oil, or that of succulents, or of yeast, can be quantitatively demonstrated without any special skill in chemical methods.

As is evident from Fig. 1, the apparatus consists of three main parts: a thick-walled filtering flask, A (of 250–300 c.c. capacity); a burette, B; and a wide glass tube, C, for which a second burette may be used although it need not be callibrated. These three parts are connected with rubber tubing as shown.

The principle of working is to introduce the respiring material into the flask A and measure the volume of gas in the apparatus. A period is then allowed to elapse while the material is respiring and the volume of gas is again measured; the CO_2 present is absorbed with NaOH solution and the volume of gas measured once more. To allow for the changes in temperature and atmospheric pressure that may have occurred between the readings, all values are reduced to normal temperature and pressure. These data enable one to calculate the amount of oxygen used and the amount of CO_2 evolved, and hence to obtain the respiratory quotient.

Before carrying out an experiment it is necessary to measure the combined volumes of the flask, connecting-tube, and end of the burette to the point where the graduations begin. To do this, disconnect the burette B from the rubber tubing at its lower end and reverse its position, i.e. turn it through 180°. Fill the flask with water and push in the cork to the position it will occupy in subsequent experiments. Note the height of the water in the burette. (If the level stands below the graduation marks, add water to bring the level on to the scale.) Remove the cork and pour the water into a graduated vessel: the volume of this water minus the reading of the burette gives the required volume. Call this v_1.

Prepare a small sealed bulb, *d*, containing NaOH solution and ascertain its

volume (v_2) by immersing it in a graduated cylinder of water; in a similar manner ascertain the volume (v_3) of the seeds or other respiring material to be used; then introduce both NaOH bulb and seeds into the flask, insert the cork and seal with wax. The volume of gas now in the apparatus up to the first graduation mark of the burette is $v_1 - v_2 - v_3$: call this value v.

The burette is now reversed in position and connected again with the tube c and water is poured in until it stands about half-way up B and C. (The tube *e* must be momentarily detached to allow the water to rise in B). Allow the apparatus to stand for a quarter of an hour to acquire the temperature of its surroundings, then adjust the tube C until the water stands at the same level in B and C (in order that the gas in the flask may be at atmospheric pressure) and record the reading of the burette. The total volume of gas in the system is v plus the value of the burette reading; ascertain the temperature and atmospheric pressure and reduce this volume to N.T.P. (= X c.c.).

If the respiring material possesses or is likely to develop chlorophyll, cover the flask with a black cloth. It is desirable, also, to close the clip *f* to guard against the possibility of CO_2 being absorbed by the water in the burette. As a precaution, the junction between glass and rubber tubing at *e* may be sealed with wax.

After a convenient interval, the amounts of oxygen absorbed and CO_2 evolved during respiration are measured in the following way: The clip *f* is opened and the tube C adjusted to bring the water in it and in the burette to the same level. The total volume of gas now in the apparatus is v + the new burette reading. Ascertain the temperature and atmospheric pressure and reduce this volume to N.T.P. (= Y c.c.).

Now break the NaOH bulb by shaking the flask, allow time for any CO_2 present to be absorbed, adjust the water-levels, and from the burette reading calculate the volume of gas in the apparatus at N.T.P. (= Z c.c.).

Then the oxygen absorbed = X − Y; and the CO_2 evolved = Z − Y.

In shaking the flask to break the NaOH bulb, care should be taken to handle the flask as little as possible as the heat from the hands will cause the gas in the flask to expand. The fifteen minutes or so which must be allowed to elapse after breaking the bulb to ensure complete absorption of the CO_2 is a safeguard against error from this cause.

When used in this simple way, the apparatus has been found to give perfectly satisfactory results in the hands of students. The amount of respiring material should obviously be correlated with the time interval between the first and two subsequent readings; with too little material or too short a time the volume changes are small and less easily measured, while with too much material for the time interval used, the volume changes are so large that the water-level in the burette is off the scale if the water-levels are equalized, and one is put to the inconvenience of measuring the difference between the water-levels and calculating the resultant lessening of pressure below that of the atmosphere. Three pea seeds or twenty-five rape seeds have been found to produce changes of volume convenient for measurement in two or three days at an average temperature of about 18° C., while 25 c.c. of a yeast suspension in sugar give reasonably large volume changes in a couple of hours or less.

An obvious shortcoming of the method as described is that the respiring material comes in contact with the NaOH and is killed, so that only the one set of measure-

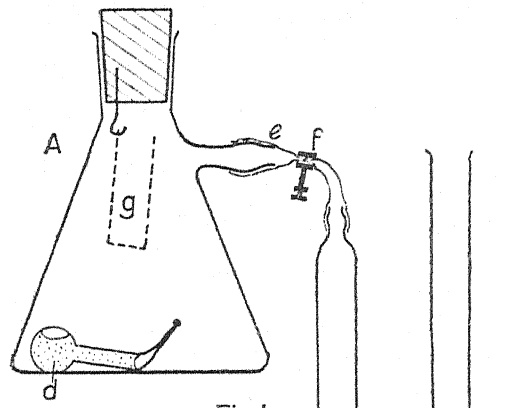


Fig.1

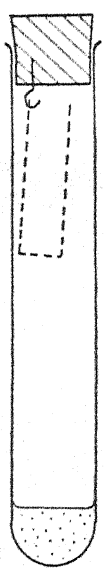


Fig.2

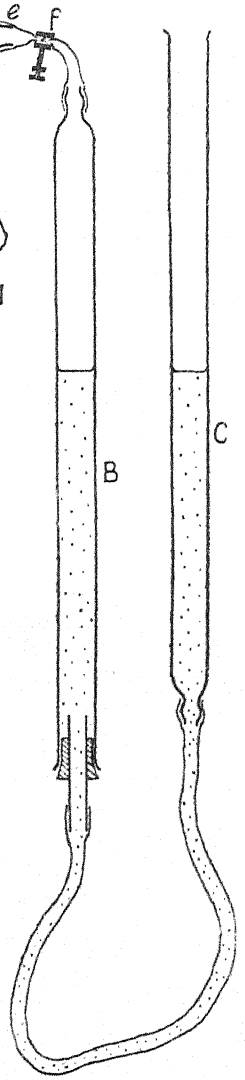


FIG. 1. Apparatus employed in Method I. A, germination flask; B, burette; C, adjustable tube for equalizing water-levels; *d*, NaOH bulb; *e*, junction of rubber tubing to side arm of flask; *f*, clip; *g*, perforated zinc foil container for respiring material.

FIG. 2. For description see text (p. 845).

ments is possible. If records are required from the same material in successive periods, the modification shown in the figure may be adopted. In this case the seeds (or other respiring material) are held in a small cylindrical container constructed of

perforated zinc foil and suspended from a wire hook in the cork of the flask. After one set of readings have been taken, the cork is removed, the flask washed out, and the apparatus set up afresh. In the case of germinating seeds it is desirable to include a piece of wet filter paper in the container to ensure a plentiful supply of water to the seedlings. The volume, v_2, of each NaOH bulb can be measured before insertion, although the error arising from small variations in size between the bulbs is very small. The largest errors are likely to occur undoubtedly over the temperature measurements, especially from sudden temperature changes affecting the thermometer and gas in the flask differentially. If results as precise as possible are required it is desirable for the bulb of the thermometer to be within the flask and the flask to be surrounded by some non-conducting material to prevent sudden fluctuations in temperature of the surroundings being transmitted to the gas in the flask. However, for ordinary purposes this complication seems unnecessary if care be taken to avoid draughts, the proximity of lighted burners, direct sunshine, &c.

To illustrate the type of result obtainable with the apparatus it may be mentioned that the average value for the respiratory quotient of germinating pea seeds obtained by a class of students was 1·03, the extreme values ranging from 0·99 to 1·08; while the average value obtained from oily seedlings a few days old was 0·5 and from yeast 2·54.

The accompanying graph illustrates the course of gaseous exchange of rape seedlings during the first ten days of growth. Fifty seeds were used, and the oxygen absorbed and CO_2 evolved during 24-hour periods measured (on three of the days no measurements were taken). The experiment was carried out under ordinary class conditions with the thermometer outside the flask, and no doubt had the precautions against errors in temperature readings suggested above been taken, smoother curves would have been obtained: but information was desired as to the kind of result yielded under the simplest conditions. The readings show quite clearly: (1) the gradual increase in the amount of CO_2 evolved during germination and growth; (2) the high oxygen consumption in the early stages of germination when fats are undergoing oxidation—even during the first twenty-four hours after wetting there is a large oxygen consumption, although no measurable amount of CO_2 is evolved; (3) the gradual approach towards unity of the respiratory quotient, i.e. towards equality in the volumes of oxygen absorbed and CO_2 evolved. (See Fig. 3.)

Method II.

The change in H-ion concentration that may be brought about as a result of evolution of CO_2 during respiration, and the recognition of this change by the use of an indicator dye has been employed by a number of workers as a means of following the course of respiration in plants. But it is probably not generally recognized that the principle may be employed in an extremely simple and effective way for class purposes if comparative results only are required. The following method has been found to give satisfactory results.

To about a litre of distilled water add a few drops of brom-cresol-purple so that the solution is a pale but easily recognizable colour when examined in a wide (1″) test-tube. This solution is divided into two parts: to one a few drops of lime water are

added, to the other a little standard buffer solution having a pH value of about 6·2 (e.g. Sørensen's phosphate mixture, $Na_2HPO_4 \cdot 2H_2O$ two parts to KH_2PO_4 eight parts). Enough of each of these standard solutions is poured into two wide test-tubes (7″ × 1″) to occupy about an inch of the length.

The test-tube containing the buffered solution is corked and kept as a colour standard; the other is used for the detection of the CO_2 evolved from the experi-

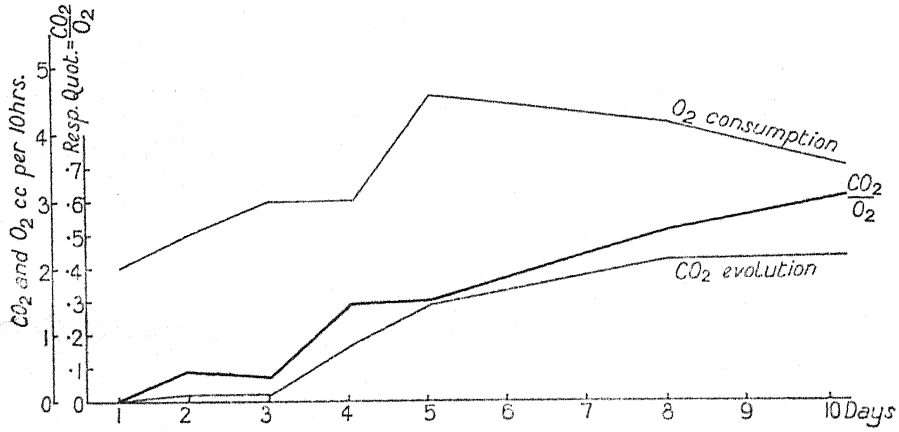


FIG. 3. *Respiration of germinating rape seeds.* 50 seeds were used: CO_2 and O_2 are represented as volume in c.c. at N.T.P. evolved or consumed in ten hours. Note large excess of oxygen consumption over CO_2 evolution in early stages with subsequent approach to equality.

mental material. If the concentration of CO_2 be increased, as for example by breathing into the test-tube, and the liquid shaken, some of the calcium carbonate is converted into bicarbonate, the resulting increase in H-ion concentration being indicated by the solution becoming more yellow: if the concentration of CO_2 is diminished, as may be accomplished by blowing CO_2-free air through the solution, the bicarbonate reverts to carbonate and the solution becomes purple owing to the reduced H-ion concentration.

The respiring material may be conveniently held in a small cylindrical container made from perforated zinc foil and hung from a wire hook in the cork of the test-tube as described on p. 843 for the other piece of apparatus (see Fig. 2). If half a dozen germinating pea seeds are put in the container and corked up in a test-tube with the purple solution, the colour changes gradually to yellow as the concentration of CO_2 in the test-tube rises. The liquid should be agitated by shaking the test-tube at frequent intervals to bring the liquid in contact with the gas in the tube and facilitate the solution of the CO_2, care being taken that the liquid does not come in contact with the container. The time taken for the solution to change in colour to the standard tint of the buffered solution in the other test-tube may be taken as a measure of the intensity of respiration. With half a dozen peas this should be in the neighbourhood of half an hour at the ordinary laboratory temperature.

Having carried out an observation as described, the cork is removed and air blown through the solution, now yellowish in colour, from a scent spray bulb attached to a

piece of glass tubing long enough to reach to the bottom of the test-tube, when it will rapidly resume the purple colour and a fresh observation may be taken. It is not necessary to use air freed from CO_2, since the concentration of CO_2 in ordinary air is too low to be of significance in altering the colour of the solution.

By this method the effect of conditions such as temperature, anaesthetics, &c., on the rate of respiration can be readily demonstrated. If a green leaf be used the effect of light on CO_2 output may be shown, the solution changing from purple to yellow in the dark or in a dim light, and back to purple when the leaf is brightly illuminated.

In conclusion, it should be noted that the brom-cresol-purple solution should not be too strong, as strong solutions are dichroic and difficult to match with a standard.

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June, 1929.